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SELF-INCOMPATIBILITY IN RYE

II. GENETIC CONTROL IN THE TETRAPLOID

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I. INTRODUCTION

As demonstrated previously (LUNDQVIST, 1954, 1956), self- and cross-incompatibilities in rye are controlled gametophytically by two apparently independently segregating loci, designated the S locus and the Z locus, each probably with a series of multiple alleles. The pollen from the diploid self-incompatible plant will accordingly carry two incompatibility factors, one for each of the loci, and the reaction of the pollen is determined by its own genotype. In the material analysed (LUNDQVIST, 1956), pollen with one factor common to the style had no functional disadvantage, irrespective of the locus. Thereby occurrence in the pollen of »dominance» factorial interaction between the loci could be ruled out, and, thus, both factors of the pollen had to be matched in the style in order to produce inhibition of pollen tube growth. Thus, the factors of the pollen did not act independently of each other. Several ways of obtaining such a functional dependence between the two incompatibility factors of the haploid pollen are conceivable:

(1) The factors are capable of two reactions, opposed to one another: unlike factors in pollen and style, causing a positive promotion of pollen tube growth; like factors, causing a positive inhibitory reaction: a promotive reaction always dominating an inhibitory reaction.

(2) A passive co-operation between the factors, each matched factor independently producing an immunity reaction that will not reach alone a critical threshold strength below which a pollination appears fully compatible.

(3) An active and regular co-operation between the factors, the specific incompatibility substances resulting from interactions between the two loci.

It has been pointed out (LUNDQVIST, 1954) that the presence of two incompatibility loci constitutes a genetical equivalent to certain cyto-

logical observations, interpretable as indicating partial polyploidy in rye (PATHAK, 1940; LEVAN, 1942; LIMA-DE-FARIA, 1952). HAYMAN (1956) discussing an identical case of two-loci control of incompatibility in another grass species, *Phalaris coerulescens* DESF., considered it more probable that the two loci are of a non-duplicative origin in view of the break-downs and impairments of gametophytically controlled self-incompatibility, known to obtain, when more than one incompatibility allele occurs together in the pollen. However, the increase of general cross-compatibility and the stability against loss of incompatibility alleles by random drift — both inherent in the multiallelic biserial system when, in the haploid pollen, a single factor matched is not capable alone of producing more or less effective inhibition of pollen tube growth — may give such a system a selective advantage over the single-locus gametophytic system (LUNDQVIST, 1954, 1956; *cf.* also BATEMAN, 1952). Accordingly a selective rehabilitation of an incompatibility system at the biserial level is far from being inconceivable after a factorial duplication that in the beginning caused impairment of the previous incompatibility system.

To understand the evolution of the present incompatibility system, the question of the origin of the second incompatibility locus — duplicative or non-duplicative — is important. Knowledge of the way of action and co-operation of the incompatibility factors in the grasses in question may give some help when inquiring into the nature and origin of these factors. Here, for two reasons, an investigation of the incompatibilities in autotetraploid rye was considered likely to give valuable information. (1) In the diploid pollen there are four incompatibility factors, and new proportions of pollen factors matched in the style that are not possible at the haploid level ($^1/_4$ and $^3/_4$) may therefore be realized. (2) Each incompatibility locus being represented by two factors in the diploid pollen, an opportunity is given here for allelic interaction within the loci also to take place.

Referring to tetraploid plants with 1, 2 (two+two, and one+three, respectively), 3, and 4 different alleles at the incompatibility locus in question, the terms *monoallelic*, *diallelic* (balanced and unbalanced, respectively), *triallelic*, and *tetraallelic* (ATWOOD, 1944), will be used in this paper. Referring to diploid pollen with two incompatibility alleles, the terms *homogenic* and *heterogenic* have been coined (FISHER, unpublished, LEWIS, 1947), the pollen having two identical alleles and two different alleles, respectively. It is suggested here that in the case of *two* incompatibility loci the terms *homoalleles* and *heteroalleles*

should refer to factors belonging to the same locus in the pollen (the pollen being *homoallelic* and *heteroallelic*, respectively), the previous terms homogenic and heterogenic being reserved for the case of factorial identities between the two loci.

Owing to the considerable complications and the less clear-cut ratios that may ensue from a tetrasomic segregation involving two loci, the study would preferably require chromosome doubling of known diploid genotypes, and an analysis of pollen tube growth together with the proportions of compatible and incompatible pollen grains in suitable genotype combinations, where the proportions of the different pollen genotypes could be calculated in advance. The primary products of doubling being balanced diallelics and/or monoallelics, complications are avoided here that would follow from double reduction in other genotypes, and only 3^2, 3 or 1 pollen genotypes can in any case be formed. The method of studying incompatibility inter-relationships by means of pollen tube growth was quite successfully employed in grasses by HAYMAN (1956), when studying diploid *Phalaris coerulescens*.

Owing to the difficulty of obtaining and conserving in the annual rye a group of known genotypes suitable for doubling, the following tentative study of seed-setting and incompatibilities in S_1 families derived from tetraploid élite population plants of unknown constitution might still be of a certain interest in combination with some quite fragmentary data from a group of known doubled genotypes and seed-setting data from several years' selfing in diploid and corresponding autotetraploid élite populations.

II. MATERIALS AND METHODS

Data about the tetraploid strain of Steel-rye used here are given by MÜNTZING (1951). The strain has been derived after colchicine treatment in 1940 from seven plants and has been subject to rigid selection for increased fertility, seed-setting now being about 75 % of that obtained in the diploid strain. The frequency of multivalents seems to be rather high, on an average more than half the number of chromosomes and up to all being associated in multivalents in the meiosis of one plant studied in detail, and about one third of the pollen tetrads being irregular in eight other plants studied (MÜNTZING, *l. c.*). Previous studies (LUNDQVIST, 1947) have shown the tetraploid strain of Steel-rye to be strongly self-incompatible to almost the same degree as that characterizing the diploid strain, seed-setting in the field after spontaneous

self-pollination in parchment bags averaging about 4.0 %, and 80—85 % of the plants being below the limit of 5 % seed-setting.

Materials of three kinds have been studied in the present investigations. In order to get seed for an inbreeding study, selfing was practised for several years since 1945 in élite population plants, which had been grown in the field without previous control of the chromosome numbers and spaced at 10 cm. intervals in rows 15 cm. apart. Parchment bags were used, in which one to several ears of a plant were isolated, pollination occurring spontaneously. Seed-setting was determined in relation to the number of isolated flowers.

In the investigation of incompatibilities in intra-family tests the previously described technique was employed (LUNDQVIST, 1956), the sib intercrosses thus being made in the greenhouse on plants transplanted from the field to pots, and including emasculation and manual pollination after microscopic control of the quality of the collected pollen.

The main material consists of four S_1 families derived from selfing of tetraploid élite population plants. Chromosome numbers of the S_1 plants were controlled in root-tip fixations. If possible, a cross combination comprised one ear with 60 pollinated flowers, but owing to lack of pollen the number of pollinated flowers was often smaller. Replications were not possible owing to the insufficient degree of tillering. For this material the environmental conditions were not favourable. The four S_1 families were sown in boxes quite late in the autumn of 1954, and not until the beginning of April 1955 were they transplanted to the field in order to obtain the uniform tillering desired. Tillering being retarded, the young ears were severely attacked by wheat blossom midges (probably *Contarinia tritici* KIRBY), and at the time of flowering in July the temperature was quite unusually high. The anthers did not develop well, anthesis often being highly disturbed: and seed-setting might also have been seriously influenced by the *Contarinia* larvae feeding in the flowers. These obstacles to seed-setting might not be so serious in the cross combinations, the larvae being removed on emasculation, and controlled pollen being applied to the stigmas; but in the spontaneously pollinated selfed ears, in any case, seed-setting was apparently negatively affected. For various other reasons the classification of mating results from seed-setting turned out to be difficult here.

Finally, in 1955, diploid families obtained from sib intercrosses ho het × het het (single heterozygote × double heterozygote), *viz.* plants

3×4 and 10×11 in family 1 (*cf.* LUNDQVIST, 1956, Table 1 and Fig. 6 and terminology) were treated with 0.25 % solution of colchicine at the coleoptile stage, the surviving plants afterwards being intercrossed. Pollen size was inspected, and ears with predominantly big pollen grains were only combined *inter se*. Incompatibility inter-relationships were assessed from seed-setting, and direct chromosome counts were postponed to the resultant progeny.

III. EXPERIMENTAL RESULTS

1. Selfing in élite populations

As frequently demonstrated for other grass species, self-incompatibility is not absolute in rye. Data bearing on the amount of this pseudo-self-compatibility in rye will be discussed in detail elsewhere, as will also the occurrence of true self-compatibility. Here the discussion of these phenomena will be restricted to those points that are particularly relevant to the way of action of the incompatibility factors.

Data from seed-setting upon selfing in tetraploid and diploid élite populations of Steel-rye in 1947—1952 are summarized in Table 1, as are also the corresponding values from open flowering. The numbers of observations varying considerably from year to year, it has been considered more convenient to tabulate the frequencies in per cent of the total numbers of observations, but the statistical calculations are naturally based on the original data.

Upon open flowering the tetraploids are less fertile, seed-setting being 80—90 % of that obtained in the diploids. Upon selfing, self-incompatibility is pronounced in all populations studied, 80—85 % of the tetraploids and about 95 % of the diploids being found below the limit of 5 % seed-setting. Seed-setting is higher throughout in the tetraploids, the small differences between the two groups being significant (χ^2 analysis no. 9, a comparison of the »lowest» tetraploid series with the »highest» one of the diploid group reveals a nearly significant difference). Within both groups there are small but highly significant differences between different populations (χ^2 analyses 1 and 5), the populations of 1950 and 1952 giving the highest values for seed-setting on selfing (χ^2 analyses 2 and 6). The variation between populations is considerably greater in the tetraploid group, the tetraploid values for χ^2 being higher throughout.

Self-incompatibility is, thus, still pronounced in the tetraploid strain,

TABLE 1. *Seed-setting upon selfing and open flowering in tetraploid and diploid élite populations of Steel-rye.*

Frequencies given in per cent of total number, but statistical analysis made on the absolute frequencies.

		Year	Per cent seed setting																				Total numbers of plants	Average of seed-setting
			0	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100	
Self-pollination	Tetraploids	1947	83.3	8.9	3.3	1.9	1.1	1.1	0.4															269	4.17
		1948	87.0	9.5	2.4	1.1																		461	3.38
		1949	84.8	9.8	2.5	2.1	0.4	0.4																244	3.73
		1950	32.4	22.6	15.5	8.9	7.2	5.4	2.6	2.3	2.3	0.5	—	0.3										349	12.20
		1952	65.7	19.6	6.3	5.6	1.4	1.4																143	5.58
	Diploids	1947	98.5	0.4	—	0.4	—	—	—	—	—	—	—	—	—	0.7								270	3.06
		1948	97.5	0.9	0.5	0.3	—	0.2	0.1	0.2	—	—	—	—	0.1	—	0.2							644	2.98
		1949	96.3	3.1	—	0.6																		160	2.75
		1950	92.5	5.1	1.0	0.4	—	0.3	—	—	0.4	0.3												294	3.28
		1952	93.3	5.0	—	1.7																		120	3.01
Open flowering	Tetraploids	1947	0.3	—	—	—	0.9	0.6	2.4	3.0	4.2	4.7	12.5	18.1	20.4	13.6	10.1	5.6	2.4	0.9	0.3			337	59.91
		1948	0.7	0.1	0.2	0.1	0.6	0.6	1.0	2.6	2.6	4.6	9.0	13.4	17.5	18.9	15.9	9.7	2.0	0.4	0.1			1098	62.22
		1949	0.2	0.3	0.3	0.4	0.3	0.8	0.9	1.5	2.4	3.5	6.8	8.1	12.9	18.5	19.5	15.9	6.5	1.1	0.1	0.0		2397	65.62
		1950	0.2	0.1	0.2	0.1	0.2	0.7	1.3	1.2	2.3	2.9	5.6	8.3	15.3	18.2	19.0	16.3	6.2	1.7	0.2			2421	66.28
	Diploids	1947					0.2	0.2	1.4	1.1	1.6	1.8	3.2	4.6	4.8	8.2	16.0	16.0	18.0	12.5	7.7	2.5	0.2	439	69.92
		1948	0.6	0.1	0.1	0.1	0.1	0.3	0.2	0.5	0.5	0.7	1.2	2.1	3.1	5.7	9.6	17.9	24.5	21.6	9.7	1.4		1560	78.86
		1949	0.5	0.2	0.3	0.2	0.4	0.5	0.7	0.7	1.4	1.5	3.6	5.8	8.9	13.9	15.8	17.4	16.7	8.8	2.6	0.1		1624	71.22
		1950	0.0	0.3	0.1	—	0.1	0.1	0.2	0.2	0.4	0.4	0.7	1.1	1.9	3.5	6.3	15.4	22.4	28.5	15.6	2.8		2263	82.22

Table 1 (continued). χ^2 analyses of seed setting upon selfing

		Differences	χ^2	d. f.	P	Classes pooled
1.	Tetraploids	Total material	445.4	16	0.001	20—100
2.		1947/1950	162.7	4	0.001	20—100
3.		1947/1948	9.1	3	0.05—0.02	15—100
4.		1950/1952	56.7	6	0.001	30—100
5.	Diploids	Total material	16.3	4	0.01—0.001	5—100
6.		1948/1950	13.0	1	0.001	5—100
7.		1948/1949	0.771	1	0.5—0.3	5—100
8.		1950/1952	0.084	1	0.8—0.7	5—100
9.		Tetraploids 1948/ Diploids 1950	5.8	2	$\leqslant 0.05$	10—100

TABLE 2. *Frequencies of seed-setting upon selfing in diploid and tetraploid S_1 plants, derived in 1947—1949 from élite parent plants with less than 5 % seed-setting upon selfing.*

	Per cent seed-setting																		Number of	
	0	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	plants	families
Tetraploids	459	39	9	6	3	1	2	1	—	—	—	—	—	1						521	296
Diploids	345	13	7	3	4	6	5	4	2	5	2	5	7	2	1	2	—	1		414	256

but on an average the tetraploids set more seed upon selfing than do the diploids. The degrees of fertilization in the selfed materials are not exactly known, and considering the increased kernel abortion after selfing, observed in a diploid variety investigated by LANDES (1939), it might be argued, as actually done by PLARRE (1954), that the kernel abortion is an inbreeding effect due to homozygosity, more easily attained at the diploid level, and that, accordingly, the tetraploids should indeed be expected to give the higher seed-setting upon selfing. On the other hand kernel abortion due to aneuploidy may be much more frequent at the tetraploid level. HÅKANSSON and ELLERSTRÖM (1950) observed that, upon cross-pollination, abortion of fertilized embryo sacs is more frequent in the tetraploid than in the diploid strain of Steel-rye. The degrees to which these two causes of kernel abortion will be able to affect seed-setting after selfing in diploid and tetraploid rye are not known, but as in the present investigation quite small, early aborted seeds have also been taken into account, it seems justifiable to conclude here that fertilization after selfing really occurs more easily in the tetraploids. This weakening of self-incompatibility may be fortuitous, due to the narrow genetic basis of the tetraploids, or it may be a recombination effect only possible at the tetraploid level. However the same tendency was also observable in the much younger tetraploid strain of Toivo rye (LUNDQVIST, unpublished), and in the tetraploid strain of Petkus rye (PLARRE, 1954), which has a fairly broad genetic basis (SENGBUSCH, 1940). It therefore seems probable that the observed weakening of self-incompatibility is an intrinsic property of polyploidy.

It is not probable that the considerably higher variability in seed-setting between tetraploid populations of different years is due to genetic drift, although the young tetraploid strain may not have reached an equilibrium adapted to the selection applied. At the tetraploid level the average gene frequencies will be higher and the genetic drift less intense; and these tendencies will be strengthened by the narrower genetic basis of the tetraploid strain. The incompatibility reaction in the tetraploids is apparently less effectively buffered against external influences. The increased values of seed-setting, obtained upon selfing in both populations of 1950, might reflect such an influence. During the time of flowering in 1950 the temperature was quite low, although not seriously interfering with life processes, but it might have been sufficiently low to cause a delay of the incompatibility reaction, such as demonstrated by LEWIS (1942) in *Oenothera organensis*, *Prunus avium*, and *Primula obconica* and *sinensis* at certain temperatures below that which is nor-

mal to the species. The seed-setting is considerably more increased in the tetraploid population. Also in 1952, temperature during flowering was rather low.

The frequency of self-compatibility seems to be different in the two groups of populations. It will be found from Table 1 that under normal conditions (populations 1947—1949) some few diploid plants are obtained which give a reasonably »full» seed set upon selfing, whereas among the 974 selfed tetraploid plants none is obtained. In the diploid S_1 generation the frequency of plants highly fertile upon selfing is considerably increased, and now, but much more rarely, tetraploid plants of the same kind are obtained. In most cases this high fertility upon selfing is retained in later S generations. Further data to be considered elsewhere make it probable that this suddenly increased fertility is not always due to segregating modifiers, affecting the incompatibility reaction, but may result from a loss mutation at an incompatibility locus. Table 2 shows the frequencies of various degrees of seed-setting upon selfing in diploid and tetraploid S_1 plants, derived from population plants with less than 5 % seed-setting upon selfing. From Table 2 alone it is impossible to judge which of the plants are truly self-compatible, but in any case such plants must be considered to occur considerably less frequently in the tetraploid material. It will be evident at a later stage that this fact is of considerable importance for the evaluation of the incompatibility control at the level of the diploid pollen.

Thus the four incompatibility factors in the diploid pollen, always common to the style upon selfing, will invariably render the pollen incompatible, though on an average less efficiently than do the two matched factors of the haploid pollen. Whether there are differences in the strength of the incompatibility reaction, when the selfed diploid pollen is homoallelic or heteroallelic (at one or both loci) cannot be decided from the present material. In any case, the incompatibility reaction must be considered strong.

2. Intercrosses of doubled genotypes obtained from sib intercrosses ho het×het het

The genotypes of the intercrossed diploid siblings and the resultant progeny may be written:

Parents:		$S_{1.1}Z_{3.4}\times S_{1.2}Z_{3.4}$	
Diploid offspr. fam.:	$S_{1.2}Z_{3.3}$	$S_{1.2}Z_{4.4}$	$2\,S_{1.2}Z_{3.4}$
Doubled genotypes:	$S_{1.1.2.2}Z_{3.3.3.3}$	$S_{1.1.2.2}Z_{4.4.4.4}$	$S_{1.1.2.2}Z_{3.3.4.4}$

Diploid siblings, not affected by the colchicine treatment, segregated according to expectation in three groups showing the expected incompatibility inter-relationships (5 ho_ahet, 2 ho_bhet, 11 het het; χ^2 for homozygotes to heterozygotes=0.88, and P=0.5—0.3). The four remaining plants, apparently affected, were completely self-incompatible, and only one intercross combination was successful, 21 seeds being obtained from 72 pollinated flowers. 19 seedlings were obtained, all of them tetraploid.

TABLE 3. *Genotypes of diploid pollen formed from the double diallelic* $S_{1.1.2.2}Z_{3.3.4.4}$, *and corresponding numbers of alleles matched in the style of* $S_{1.1.2.2}Z_{3.3.3.3}$.

No.	Pollen genotypes	Alleles matched		Number of matched alleles	
		S	Z	Totally	Different alleles
1	$S_{1\ 2}Z_{3.3}$	$S_{1.2}$	$Z_{3.3}$	4	3
2	$S_{1.1}Z_{3.3}$	$S_{1.1}$	$Z_{3.3}$	4	2
3	$S_{2.2}Z_{3.3}$	$S_{2.2}$	$Z_{3.3}$	4	2
4	$S_{1.2}Z_{3.4}$	$S_{1.2}$	Z_3	3	3
5	$S_{1.1}Z_{3.4}$	$S_{1.1}$	Z_3	3	2
6	$S_{2.2}Z_{3.4}$	$S_{2.2}$	Z_3	3	2
7	$S_{1.2}Z_{4.4}$	$S_{1\ 2}$	—	2	2
8	$S_{1.1}Z_{4.4}$	$S_{1.1}$	—	2	1
9	$S_{2.2}Z_{4.4}$	$S_{2.2}$	—	2	1

The two combined plants being only one way compatible, the male compatible plant, most probably, was the double balanced diallelic $S_{1.1.2.2}Z_{3.3.4.4}$, the female compatible plant belonging to one of the single balanced diallelic genotypes, say $S_{1.1.2.2}Z_{3.3.3.3}$. The nine pollen genotypes possible from the tetraploid double balanced diallelic plant, when applied to the style of the single balanced diallelic plant in question, will have two, three, or all four of its incompatibility alleles matched in the style (Table 3). At least one of these genotypes must have been able to react compatibly. Data from selfing, considered in the previous section, indicate that the pollen genotypes Nos. 1—3 will react incompatibly, as is also suggested from the presumed single balanced diallelic plant being incompatible as male on the double heterozygote. Data to be considered in the following section demonstrate that, at least sometimes, pollen

with three of its four factors matched in the style, such as genotypes Nos. 4—6, may react incompatibly. In the remaining pollen genotypes Nos. 7—9, the two matched factors belong to the same locus, the locus being heteroallelic or homoallelic. Judging from the reactions of haploid pollen, the present homoallelic pollen genotypes Nos. 8 and 9 will most probably react compatibly, whereas the reaction of No. 7 cannot be suggested. Direct experiments are carried on to test critically the reaction of such heteroallelic pollen.

Thus the conclusion seems justifiable that two homoalleles of the pollen, matched in the style, will not be able alone to render the pollen incompatible.

3. Incompatibilities in S_1 families

The material studied is presented in Table 4. In the autumn of 1951, 24 tetraploid plants taken from an élite population were cloned, and most of the cloned plants could be selfed in two subsequent years. Owing to lack of time, the S_1 families sown out in the autumn of 1952 were not tested, neither to their respective parent plant, nor in a similar intercrossing scheme as were corresponding diploid S_1 families of Steel-rye (see LUNDQVIST, 1954); their self-incompatibility status, however, was tested by means of selfing. The four S_1 families studied in 1955 are derived from population plants of intermediate or rather weak (family 441) self-incompatibility.

a. Selfings

It is evident from Table 4 that all the 24 population plants selfed are clearly self-incompatible, the population apparently being typical of the populations previously studied. There is a good agreement within different clones between the values of seed-setting on selfing from the two years ($r = +0.87$; $P < 0.001$). Thus the different degrees of self-incompatibility are, obviously, only to a small extent conditioned by external influences. Selfings of plants in the S_1 families of 1953 stress this conclusion, the correlation between S_1 families and their respective parent plant for seed-setting on selfing in 1953 corresponding to a coefficient $r = +0.50$ ($P \geq 0.05$). As can be seen from Table 5, no self-compatible S_1 plants were obtained, neither in 1953 nor in 1955, among the 124 plants selfed, such as were obtained among 155 diploid S_1 plants belonging to 15 different S_1 families (see LUNDQVIST, 1954, Table 1). The lower seed-setting values from S_1 selfing in 1955, especially evident in family

TABLE 4. *Material of tetraploid Steel-rye studied in 1952—1955.*

Selfings												Sib intercrosses 1955 $S_1 \times S_1$			
Parent plant						S_1 generation									
Clone number	Chromos. number	Flowers selfed		% seed setting		Plants selfed		Flowers selfed		% seed setting		Number of combinations			% comb. classed as compat.
		1952	1953	1952	1953	1953	1955	1953	1955	1953	1955	Total	Setting no seed	Setting seed	
431	28	1182	534	1.2	2.1	4	—	244	—	3.3	—				
432	29	626	286	1.6	0.3	5	—	444	—	8.6	—				
433	28	400	978	0.3	0.5	—	—	—	—	—	—				
434	27	736	756	0.3	0.0	—	—	—	—	—	—				
435	28	284	136	1.1	0.7	1	—	72	—	2.8	—				
436	28	—	722	—	2.1	—	12	—	1268	—	0.4	67	30	37	55.2
437	29	660	372	2.4	3.8	9	—	744	—	3.9	—				
438	29	172	—	0.6	—	1	—	74	—	0.0	—				
439	28	1680	1606	0.8	1.2	4	8	514	952	0.2	0.0	34	9	25	73.5
440	28	176	560	0.6	1.3	1	—	268	—	0.0	—				
441	28	1068	1910	7.3	17.7	4	16	592	1704	7.8	0.8	129	51	78	60.5
442	28	1040	824	3.2	3.8	3	11	522	1312	0.4	0.3	85	42	43	50.6
443	28	560	160	0.5	3.8	1	—	54	—	0.0	—				
444	28	832	1344	0.4	1.4	1	—	144	—	2.1	—				
445	28	792	—	3.2	—	7	—	810	—	3.2	—				
446	28	82	936	0.0	0.4	—	—	—	—	—	—				
447	28	2048	192	0.2	0.5	4	—	396	—	1.8	—				
448	28	870	1328	0.5	1.7	4	—	412	—	1.0	—				
449	28	1032	404	0.0	0.2	—	—	—	—	—	—				
450	28	1202	—	3.1	—	8	—	698	—	15.6	—				
451	28	324	76	1.9	5.3	—	—	—	—	—	—				
452	28	1418	538	5.4	7.4	7	—	1128	—	9.4	—				
453	28	866	770	4.7	4.0	8	—	816	—	1.8	—				
454	28	1194	1638	0.8	2.9	5	—	316	—	0 9	—				
Total				1.74	2.91					3.49					

TABLE 5. *Seed-setting upon selfing and crossing within four selected clones of tetraploid rye and the corresponding S_1 families. Each value corresponds to one ear.*

× = crossing ⊗ = selfing

Parent clone No.	Generation	Percent seed-setting																					
		0	0	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100
436	⊗ Clone 1953	4	4	1																			
	⊗ S_1 55	8	5																				
	× » 55	30	6	2	1	3	2	4	2	1	2	3	3	1	3	2	1	—	—	1			
439	⊗ Clone 1952	12	10																				
	⊗ » 53	10	9																				
	⊗ S_1 53	7	1																				
	⊗ » 55	12																					
	× » 55	9	10	2	—	2	3	1	3	2	1	—	1										
441	⊗ Clone 1952	1	5	2	3	1																	
	⊗ » 53			3	9	2	5	3	—	1													
	⊗ S_1 53		2	3	—	2																	
	⊗ » 55	10	8																				
	× » 55	51	8	10	16	11	9	7	5	3	2	1	4	—	2								
442	⊗ Clone 1952	2	9	—	—	—	1																
	⊗ » 53	1	9	1	1																		
	⊗ S_1 53	6	2																				
	⊗ » 55	11	2																				
	× » 55	42	14	6	10	5	3	2	—	1	2												

441, are probably due to wheat blossom midges and poor pollen production (see above), but the unusually high temperature prevailing during flowering might moreover have rendered the self-incompatibility reaction faster and the incompatibility sieve more effective (*cf.* LEWIS, 1942).

b. S_1 sib intercrosses

The pollination schemes with seed-setting values of the four S_1 families 436, 439, 441, and 442 are given in Tables 6—9, and the values of seed-setting are also summarized in Table 5. In all families there is a gradual increase of seed-setting, from combinations completely failing, to values suggesting clear compatibility. The classification of mating results is rendered still more difficult, as the corresponding values from selfing are not quite reliable owing to larvae of wheat blossom midges feeding in the flowers, and to disturbed pollen production (see above). It is not probable that seed-setting from the cross combinations has been unaffected by these obstacles to seed-setting. Thus it has been considered a reasonably safe approximation to treat combinations, setting at least one seed, as compatible, whereas the completely failing combinations will be treated as incompatible. In families 439 and 442 no essential error seems to be introduced in that way, whereas families 436 and 441 may be more unreliable.

The conclusions that can be drawn from the data of the four S_1 families must necessarily be greatly limited. A genotypic classification of the tetrasomic segregation without a clear classification of incompatibilities and knowledge of the genotypes of the selfed parental plants, will be extremely unsafe; and owing to the external disturbances to seed-setting no valid conclusions from seed-set data will be possible.

When grouping together reciprocally incompatible plants, it at once becomes evident that the plants of the group do not always react uniformly with common third tester plants. This indicates primarily that, in the tetraploids, reciprocally incompatible plants may belong to different genotypes. By itself, this is not surprising. The incompatibility reaction of the diploid style being sporophytically controlled without allelic interaction resulting in dominance or mutual weakening of the alleles (LUNDQVIST, 1956), tetraploid styles carrying the same incompatibility factors should be expected to be able to inhibit the same pollen genotypes. Whereas in the diploid style the two alleles of a heterozygous locus will occur together in only one combination, the given alleles of a diallelic tetraploid can be combined in three ways (*e. g.*, $S_{1.1.1.2}$, $S_{1.1.2.2}$,

$S_{1.2.2.2}$), and those of a triallelic, also in three ways (*e. g.*, $S_{1.1.2.3}$, $S_{1.2.2.3}$, $S_{1.2.3.3}$). The point of interest is that it has been possible at all to detect lacking genotypic identity of reciprocally incompatible plants.

For the sake of simplicity of argument, only one locus will be considered in the following discussion, assuming that the incompatibility reaction ultimately depends on the conditions at this locus. To take the simplest example, it is at once evident that, without double reduction, the unbalanced diallelic plant can form only two pollen genotypes; the balanced diallelic, three. Thus, with chromosome assortment, a plant $S_{1.1.1.2}$ will form pollen $S_{1.1}$ and $S_{1.2}$, whereas the plant $S_{1.1.2.2}$ will form pollen $S_{1.1}$, $S_{1.2}$, and $S_{2.2}$. The two plants will be reciprocally incompatible. Assuming a third plant, of the genotype $S_{1.1.1.1}$, it is evident that the pollen $S_{2.2}$ from the balanced diallelic plant will be compatible here. If both S alleles of the pollen must be matched for incompatibility to be produced, then the pollen $S_{1.2}$ will also be compatible, and the two diallelic genotypes may not be distinguished from one another, when incompatibilities are assessed from seed-setting. It is evident and can be verified in more detailed combination schemes that a reaction pattern with reciprocally incompatible plants reacting differently with a common third tester plant will indicate that, for the locus in question, (1) even one allele of the pollen common to the style is sufficient for incompatibility, and that (2) double reduction does not obtain in all plants. To obtain the above reaction pattern it is not necessary that also the second incompatibility locus behaves in the same way; at least one locus, however, has to work under the conditions just mentioned.

As can be seen more clearly from Fig. 1, this interesting incompatibility pattern is obtained in all four families studied. Of special interest is the observation that reciprocally incompatible plants may react differently as female when pollinated by the same third plant. This clearly indicates that the two plants in question are not composed by the same incompatibility factors. Such a factor not common to both plants, would make the pollen carrying it compatible on the other plant, provided that the incompatible reaction of the pollen required identity in the style for all four factors. Double reduction pollen, homoallelic for this factor, would in any case be uninhibited. Thus, the conclusion is reached once more that, at least for one locus, not both factors of the pollen need be matched in the style for incompatibility to be produced, and that double reduction does not regularly obtain for this locus.

The way in which occurrence of aneuploid pollen will affect the incompatibility inter-relationships in tetraploid rye has to be considered

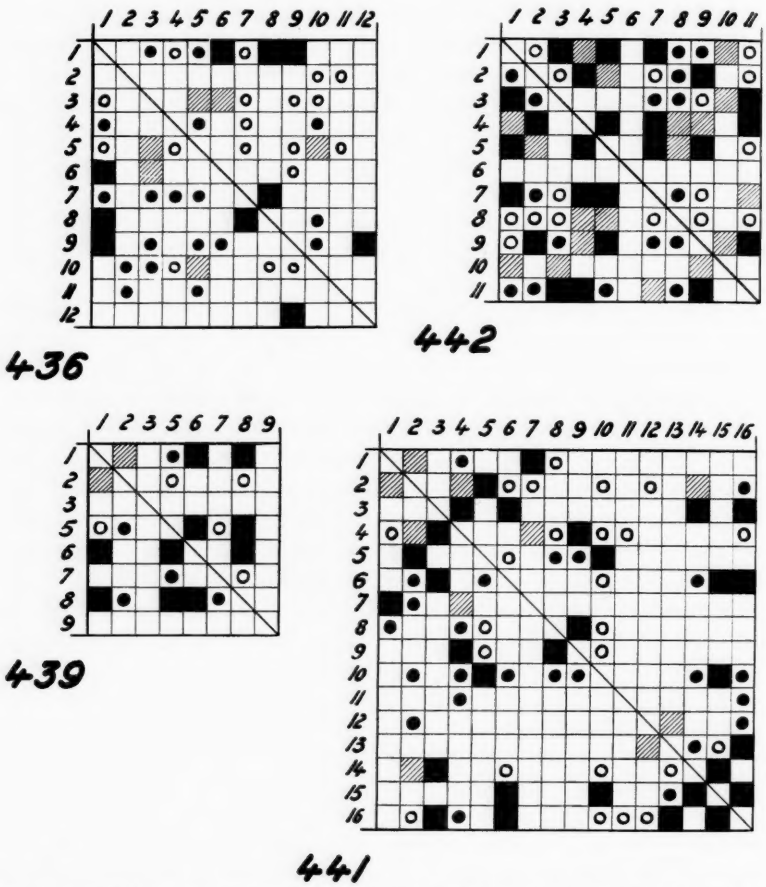

Fig. 1. Results from the reciprocal intercrosses within the four tetraploid S_1 families of Tables 6—9. Rows represent plants tested as females; columns, plants tested as males.

Symbols:

▨ plants reciprocally incompatible.

■ » » compatible.

◉ } » one way compatible → setting seed.
○ } ↘ setting no seed.

Blank: pollination not made.

here. Disregarding the functional disadvantage due to aneuploidy *per se*, it seems probable that hyperploid pollen with five incompatibility factors will display normal incompatibility reactions (the hyperploid parent plants in Table 4 not being more fertile upon selfing than the euploid parent plants), and that occurrence of such pollen will not change the incompatibility inter-relationships. On the other hand occurrence of hypoploid pollen with three incompatibility factors may affect the incompatibility inter-relationships in the same way as will occurrence of double reduction homoallelic pollen, thus leading to a uniform reaction pattern of reciprocally incompatible plants and possibly to the lack of a clear cut border-line between incompatibility and compatibility in cross combinations. The important conclusion here will be that the previous argumentation is not invalidated by the occurrence of aneuploid pollen.

It might be argued that the reaction pattern with reciprocally incompatible plants reacting differently with a common third plant could also be obtained if the single locus alone were able to produce incompatibility, independently of the other locus. As can be easily realized, however, complete inhibition of seed-setting, due to this alleged independent action of the single locus, would occur only rarely. As rendered highly probable from the data of the previous section and from evidence at the level of diploid rye, homoalleles of the diploid pollen, matched in the style, will not be able alone to render the pollen incompatible. Homoallelic pollen will always be produced, in varying proportions, from all genotypes except the tetraallelics, and a combination between two plants tetraallelic at the same locus will occur only rarely. Therefore, the influence on the incompatibilities, following from an occasional pollen inhibition independently produced by matched heteroalleles of the diploid pollen, seems to be too trivial to account for the frequent occurrence of the reaction pattern in question. But, of course, the amount of seed-setting may be influenced, the proportion of compatible pollen being reduced.

The different tetraploid style genotypes, composed by the same incompatibility factors in different ratios, must be expected to display the same reaction pattern to the various pollen genotypes. They will thus constitute an intra-incompatible group with a uniform female reaction. Upon chromosome assortment these plants may, however, give different pollen genotype outputs. Thus, a higher number of incompatibility groups may be distinguished from the male reaction. Upon chromatid assortment with double reduction the pollen genotypes from the plants in question will be the same; and if all four factors of the pollen must

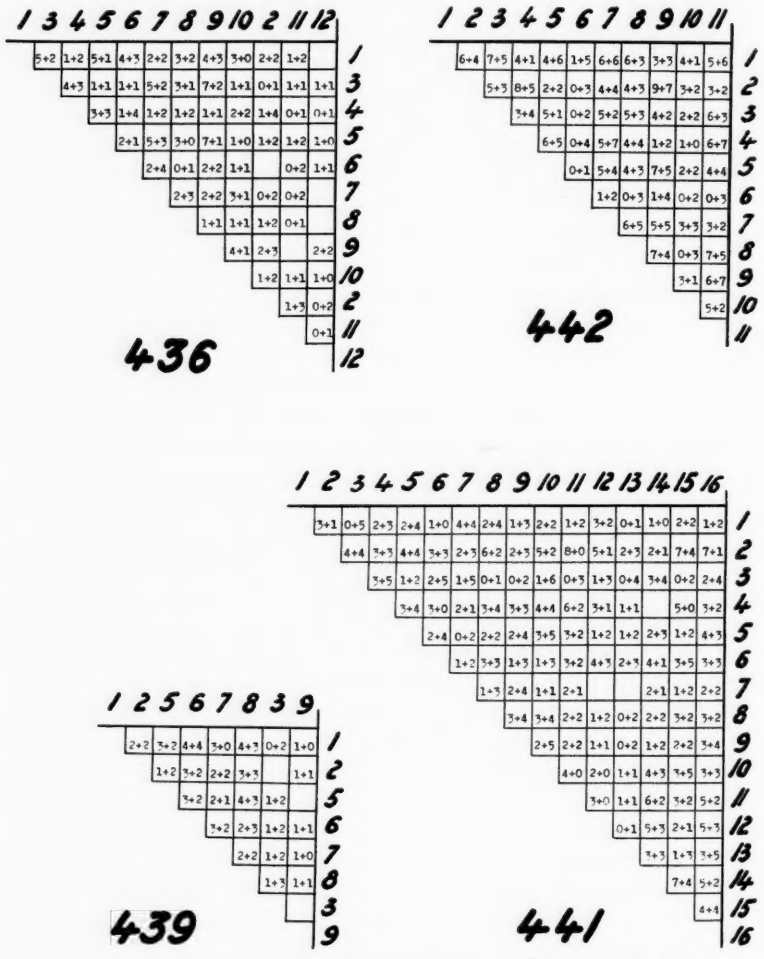

Fig. 2. The ways in which sibling plants of the four tetraploid S_1 families of Tables 6—9 react differently upon intercrosses. To the left, numbers of differing incompatibility reactions at the female side; to the right, those at the male side.

TABLE 6. *Number of seeds set/pollinated flowers from intercrosses and selfings within the tetraploid S_1 family 436.*

Rows represent plants tested as females; columns, plants tested as males. Combinations classified as incompatible, in bold types.

Chromosome no.		28	28	28	28	28	28	28	28	28	28	28	27
Plant no.		1	2	3	4	5	6	7	8	9	10	11	12
28	1	**0/96**	18/60	13/60	**0/28**	1/60	25/60	**0/56**	10/52	39/60		32/60	
28	2		**0/104**								**0/30**	**0/60**	
28	3	**0/52**		**0/110**		**0/60**	**0/22**	**0/60**	**0/60**	**0/60**	**0/30**		
28	4	15/60			**0/116**	5/60	15/60	**0/60**			14/30		
28	5	**0/60**	2/60	**0/70**	**0/30**	**2/216**	3/60	**0/60**	**0/60**	**0/40**	**0/30**	**0/60**	**0/30**
28	6	1/60		**0/60**			**1/104**			**0/56**			
28	7	22/60		17/60	1/30	31/60	13/21	**1/80**	52/60				
28	8	8/60						38/60	**0/98**		11/52		
28	9	29/60	30/60	16/60	**0/30**	19/40	22/40	25/40	19/60	**0/98**	13/20	**0/44**	6/40
28	10		1/60	1/60	**0/60**	**0/60**			**0/60**	**0/60**	**1/112**		**0/44**
28	11		28/40			8/20						**0/74**	
27	12									10/56			**0/60**

TABLE 7. *Number of seeds set/pollinated flowers from intercrosses and selfings within the tetraploid S_1 family 439.*

Rows represent plants tested as females; columns, plants tested as males. Combinations classified as incompatible, in bold types.

Chromosome no.		27	28	27	28	28	29	28	29
Plant no.		1	2	3	5	6	7	8	9
27	1	**0/68**	**0/54**	**0/60**	1/60	1/30		1/60	
28	2	**0/30**	**0/86**		**0/60**			**0/60**	
27	3			**0/102**					
28	5	**0/30**	2/60	12/60	**0/84**	1/60	**0/30**	1/60	
28	6	1/26	19/60	1/30	9/60	**0/82**		12/60	
29	7	**0/40**	1/52	3/60	23/60	18/60	**0/92**	**0/56**	
28	8	6/30	15/60	10/60	19/60	24/60	1/26	**0/354**	
29	9		23/60			5/60		32/60	**0/84**

be common to the style for incompatibility to be produced, the male reaction, when incompatibilities are assessed from seed-setting, will likewise be the same for all the members of the intra-incompatible group. In such cases, the number of male incompatibility groups will not differ from the number of groups obtained from the female reaction.

In Fig. 2 the numbers of differing incompatibility reactions in the four families are summarized from Tables 6—9, the female and the male numbers being kept apart. Differing incompatibility reactions imply, of course, that the plants in question have different incompatibility genotypes. The figure will thus show the numbers of incompatibility groups distinguished in the families, and express the reliability of the distinguishments. The data are summarized in Table 10. As can be seen here, there are no significant differences between the female and the male reaction patterns, but the highest number of distinguishments between plants is obtained from the male reaction, although fewer differing reactions are obtained here. Owing to the high number of genotypes that may be possible from a tetrasomic segregation involving two loci, real differences between the female and the male reaction patterns may be difficult to detect in the small numbers of plants studied in the four families.

Some other peculiar reaction patterns may be observed in Fig. 1, special attention being paid to the occurrence of one-way compatible

TABLE 8. *Number of seeds set/pollinated flowers from intercrosses and selfings within the tetraploid S_1 family 441.*

Rows represent plants tested as females; columns, plants tested as males. Combinations classified as incompatible, in bold types.

Chromosome no.		28	28	28	28	28	28	29	28	27	28	28	28	28	29	28	28
Plant no.		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
28	1	**2/90**	**0/30**		1/30			9/30	**0/30**								
28	2	**0/60**	**2/104**	3/30	**0/30**	5/30	**0/30**	**0/60**	4/30	**0/30**	**0/30**	**0/30**	**0/84**	**0/30**	**0/30**		1/30
28	3			**2/70**	12/30		16/30								6/31		6/36
28	4	**0/60**	**0/30**	4/30	**3/88**			**0/30**	**0/30**	2/30	**0/28**	**0/14**					**0/30**
28	5		11/28			—	**0/24**		7/30	1/30	5/30						
28	6		15/28	12/20		13/24	**0/88**	8/30			**0/26**				4/28	9/30	5/28
29	7	6/60	3/30		**0/28**			**0/80**									
28	8	8/30		**0/30**	7/30	**0/30**		**0/30**	**0/82**	2/30	**0/26**						
27	9	**0/30**			3/30	**0/28**			2/30	**1/96**	**0/30**						
28	10		5/30	3/30	4/30	5/30	3/30		4/32	4/36	**0/116**			**0/30**	7/26	1/30	5/30
28	11	6/30		**0/30**	7/30	**0/30**	8/30	1/30	**0/26**	**0/30**		**1/96**			4/24	4/24	16/30
28	12	11/30	10/30	1/30		**0/30**	1/30	**0/28**					**1/180**	**0/30**	4/30	**0/40**	11/30
28	13						4/30						**0/18**	**0/120**	2/30	**0/30**	4/12
29	14	**0/30**	**0/60**	3/30		**0/30**	**0/30**	**0/30**		2/30	**0/30**			**0/28**	**0/174**	8/58	
28	15		9/14				14/30	6/30	7/30	8/30	2/30			7/30	6/26	**0/88**	3/10
28	16	2/30	**0/16**	1/30	4/14	2/30	2/30		3/16	1/16	**0/30**	**0/28**	**0/20**	12/30	6/30	16/54	**1/124**

TABLE 9. *Number of seeds set/pollinated flowers from intercrosses and selfings within the tetraploid S_1 family 442.*

Rows represent plants tested as females; columns, plants tested as males. Combinations classified as incompatible, in bold types.

Chromosome no.		28	28	28	28	28	—	28	28	28	29	28
Plant no.		1	2	3	4	5	6	7	8	9	10	11
28	1	**1/120**	**0/60**	1/24	**0/44**	9/60	3/16	5/28	6/60	2/60	**0/16**	**0/60**
28	2	10/60	**0/102**	**0/15**	2/52	**0/60**	**0/20**	**0/60**	2/60	2/60		**0/60**
28	3	17/60	1/60	**0/92**	**0/82**	**0/60**	**0/20**	6/48	1/60	**0/60**	**0/18**	24/60
28	4	**0/60**	2/60		**0/96**	17/44		7/60	**0/60**	**0/60**		1/60
28	5	2/36	**0/60**		1/40	**0/110**		3/40	**0/60**	1/36		**0/60**
—	6						**0/88**					
28	7	2/60	3/30	**0/60**	1/60	6/60	1/16	**0/116**	8/60	**0/60**		**0/60**
28	8	**0/40**	**0/60**	**0/60**	**0/60**	**0/60**	**0/?**	**0/?**	**3/122**	**0/60**		**0/60**
28	9	**0/60**	4/60	2/60	**0/60**	17/60	3/16	13/60	9/60	**0/254**	**0/12**	4/46
29	10	**0/60**		**0/30**		**0/60**			**0/24**	**0/?**	**0/118**	
28	11	14/64	6/60	6/60	3/60	7/54	**0/16**	**0/60**	10/44	28/44		**0/94**

TABLE 10. *Numbers of differing reactions at the female and the male side within the four tetraploid S_1 families of Fig. 2, and the numbers of plant distinguishments obtained from these reaction patterns.*

		Numbers of differing reactions					Numbers of distinguishments				
		Female side	Male side	χ^2	d. f.	P	Female side	Male side	χ^2	d. f.	P
Families Nos.	436	113	103	0.46	1	0.50—0.30	51	56	0.24	1	0.70—0.50
	439	50	47	0.10	1	0.80—0.70	24	22	0.08	1	0.80—0.70
	441	290	296	0.06	1	0.90—0.80	106	109	0.04	1	0.90—0.80
	442	206	189	0.74	1	0.50—0.30	47	54	0.48	1	0.50—0.30
Total		659	635	0.44	1	0.70—0.50	228	241	0.36	1	0.70—0.50
Heterogeneity				0.92	3	0.90—0.80			0.48	3	0.95—0.90

combinations. It is self-evident that for a self-incompatible plant to be male compatible on the other plant, it must carry at least one incompatibility factor not represented in the other individual. Assuming that an incompatible reaction requires all four factors of the pollen common to the style, it is equally self-evident that one-way compatibility implies that fewer incompatibility alleles are represented in the male incompatible plant. On this assumption a regular pattern of incompatibilities must follow, with linear compatibility relationships. Thus, A → B and B → C will require A → C, and not A ← C or A ⇄ C or A ····· C, the arrow pointing to female compatibility, and the dotted line indicating reciprocal incompatibility. The latter three patterns of reaction will not be possible, unless not all four factors of the diploid pollen need be matched in the style for the pollen to react incompatibly. As can be seen from Fig. 1, the pattern A ····· C is obtained twice (439: 2, 4, and 1; 441: 4, 16, and 2); A ⇄ C is found 12 times, and in all families except No. 436, whereas A ← C is found in none of the families. The numbers are small, but they probably reflect different probabilities of obtaining the various incompatibility genotypes after selfing in the tetraploid population.

The lack of a clear-cut border-line between incompatibility and compatibility, whatever may be its reasons, has rendered the evaluation of the tetraploid reaction patterns very difficult. However, the conclusion seems fairly well established that, at least for one locus, not both alleles of the pollen must be matched in the style for the pollen to react incompatibly. For this observation to be made, it is required that double reduction does not regularly obtain for that locus. For the present material, most probably, it does not obtain at all. As to the other locus, nothing so far can be definitely stated.

IV. GENERAL CONCLUSIONS AND DISCUSSION

As suggested from the behaviour of haploid pollen, three alternative hypotheses for the control in the pollen of the incompatibility reaction in rye were outlined in the introduction. The distinction between them was not possible at the level of the haploid pollen. At the level of the diploid pollen, the present material permits of the following distinctions.

Assuming a reaction between unlike factors in pollen and style, positively promoting pollen tube growth and dominating the positive inhibitory reaction caused by like factors in pollen and style, the incompatible reaction of diploid pollen with one of its four factors unmatched

in the style, would not be expected. Admittedly, the proportions of matched to unmatched factors are here not the same as in haploid compatible pollen with one factor common to the style; but in any case the alternative has received no support from the present data.

The second alternative assumed the addition of immunity reactions produced by each matched factor of the pollen, the individual reaction not being sufficient alone to reach the threshold for the incompatibility reaction. The inefficiency of the assumed individual factor reaction of the *haploid* pollen could be thought of as resulting from competitive factorial interaction between loci in the pollen, occurring regularly and resulting in mutual weakening; or the factors might react without interaction, independently.

The present data demonstrate that, in the *diploid* pollen, three matched factors reacting may be sufficient for the incompatibility reaction. Nevertheless, the four factors of the selfed diploid pollen are apparently not able to produce an inhibition of pollen tube growth equally effective as that produced in the selfed haploid pollen, a slight increase of seed-setting upon selfing being observable in the tetraploid rye. Assuming incompatibility to result from added reactions of matched factors, it thus seems necessary also to assume the existence of some kind of dominance allelic interaction within loci in the diploid pollen. Considering the regular and strong self-incompatibility of the tetraploid strain, it seems, however, difficult to conceive the occurrence of competitive factorial interaction in the diploid pollen both within and between the loci. In such a case, up to four different ways of factorial interaction between loci, and two within loci, would be possible, as compared to only one, between loci, in the haploid pollen. Thus, there ist most probably no competitive allelic interaction in the pollen *between* loci, resulting in mutual weakening or dominance, neither at the haploid, nor at the diploid level.

On the other hand, it is not excluded that incompatibility may result from added immunity reactions, where the loci act independently of each other, possibly in combination with dominance allelic interaction *within* loci in the diploid pollen. It seems, however, that, upon this alternative, the reactions may be too easily affected by outer environmental factors to be able to ensure that two matched factors in the haploid pollen will always make it react incompatibly, whereas only one factor matched will not. In order to ensure a perfect incompatibility reaction on the basis of the biserial system, and, without reduction of general cross-compatibility, to take advantage of its greater stability

against loss of alleles due to random drift, the evolution of the biserial system seems necessarily to end in a complementary co-operation between unidentical factors.

In the haploid pollen these unidentical factors are always situated at different loci. In the diploid pollen, two alleles being obtained at each locus, the complementary co-operation between not identical factors might be developed within one and the same locus as well. It could be shown above that, most probably, two identical alleles of the same locus (homoalleles), matched in the diploid pollen, are not able alone to produce incompatibility, but it may be asked whether, if not identical (heteroalleles), the two matched alleles of the locus would be capable alone of rendering the pollen incompatible. As to the complementary co-operation between loci, it is made highly probable, but it may be asked here, how many of the four factors are thereby involved together in the production of the pollen specificities. It could be shown above (S_1 families 436, 439, 441, and 442) that, at least from one of the loci, the minimal prerequisite for incompatibility of the diploid pollen to be obtained, may be contributed, when a single allele is matched in the style. This does not mean that this factor alone will be able to produce incompatibility; co-operation between not identical factors is necessary. Considering co-operation between loci, the problem is whether the second locus must be represented by two matched alleles or only one, if the pollen is to react incompatibly.

In the present material there is no direct evidence from oppositional allelic data, bearing on the questions above. However, some important inferences may be drawn from the frequencies of self-compatible mutants obtained after selfing in diploid and tetraploid élite population materials. In the diploids the occurrence of truly self-compatible mutants, resulting from mutation at a pollen controlling part of an incompatibility locus, is directly proved, the haploid pollen with one mutated factor of that kind never reacting incompatibly (LUNDQVIST, unpublished); the nature of the self-setting tetraploids obtained after selfing is not settled. In any case it is a striking fact that, whereas the number of mutable factors is doubled upon chromosome doubling, yet the frequency of self-incompatible plants, giving rise to self-compatible mutants in the offspring after selfing, is much lower in the tetraploids. The lower frequency of self-compatible mutants obtained after selfing in the tetraploids can be easily understood from the previous considerations. In a haploid pollen, containing a mutated, »self-compatibility», factor, co-operation between matched, not identical incom-

patibility factors will no longer be possible; but correspondingly in a diploid pollen several ways of such co-operation may be left possible. A »self-compatibility» mutant factor will accordingly behave in a »recessive» way in the diploid pollen, in a »dominant» way in the haploid pollen.

Summing up the postulates:

(1) At least one of the loci must be capable of mutations, resulting in self-compatibility at the haploid level.

(2) From at least one of the loci the minimal prerequisite for incompatibility of the diploid pollen to be obtained may be contributed, when a single allele is matched in the style.

(3) At this locus double reduction does not obtain at the tetraploid level.

(4) The frequency of self-incompatible plants, giving rise to self-compatible mutants in the offspring after selfing, is considered to be considerably higher in the diploids.

Summing up the questions:

(a) Is the locus capable of mutations, resulting in uninhibited selfed pollen at the haploid level?

(b) Is it a prerequisite for incompatibility of the diploid pollen to be obtained that both alleles of the locus are matched in the style; or will one already suffice?

(c) Does double reduction, present at the tetraploid level, obtain at the locus?

(d) Can incompatibility be produced in the diploid pollen by the single locus alone, provided that its two factors are matched and not identical (heteroalleles)?

These four questions, each with two alternative answers, concern the two loci separately. Thus, for each locus 2^4 combinations of the alternatives are possible; and combining the two loci, $16^2=256$ different combination patterns will be obtained. However, for one of the loci, say the S locus, it could be demonstrated that (1) already a single allele of the diploid pollen, matched in the style, may contribute the minimal prerequisite for incompatibility, and that (2) double reduction does not obtain here. Thus, the tetraploid is practically unable to form uninhibited selfed pollen at the diploid level through mutation at the S locus, as pollen of that kind would require two coincident independent

mutations at the S locus. The presence of diploid uninhibited selfed pollen must, accordingly, be explained from (3) the Z locus being capable of »self-compatibility» mutations. Combining the two loci, only $2^3 \times 2^3 = 32$ combinations will thus be possible. The problem may now be stated as follows: Which combinations of the variables given above will permit the occurrence of tetraploid self-compatible mutants after selfing, but in a frequency below that obtained after selfing of diploid rye? Which of these combinations in their turn are biologically the most probable?

It is at once evident that, under the conditions given for the S locus, the presence of diploid mutant pollen uninhibited after selfing is primarily dependent on the conditions prevailing at the Z locus. In 8 of the 32 combination patterns it is assumed that, also at the Z locus, already a single allele, matched in the style, contributes to the diploid pollen the minimal prerequisite for incompatibility, and that, equally, double reduction does not obtain here. Under these conditions, practically no uninhibited diploid pollen will result from mutation at the Z locus, and no tetraploid self-compatible mutants will be obtained at all. It is equally evident that, if it is a prerequisite for incompatibility of the diploid pollen that both alleles of the Z locus are matched in the style, it will be of no significance whether double reduction obtains at the Z locus or not. One mutated factor at that locus will react in a »dominant» way, quite as it would in the haploid pollen, the diploid pollen always becoming uninhibited — *provided* that matched heteroalleles at the S locus are not able alone to produce incompatibility. Under these conditions, the frequency of self-compatible mutants in 4 of the 24 remaining combination patterns will be double that obtained in the diploids, and in another 4 combination patterns, the frequency will be equal to that of the diploids. In the former cases only the Z locus, in the latter cases both loci, are assumed to be capable of mutations, resulting in uninhibited pollen at the haploid level. Accordingly, the numbers of incompatibility factors, through mutation capable of rendering the selfed pollen uninhibited, will be doubled after chromosome doubling, and unchanged, respectively; it is thereby assumed that the mutation frequency of the individual factor after chromosome doubling remains unchanged.

Thus, 16 combination patterns can immediately be ruled out. From the remaining 16 combination patterns (Table 11), diploid mutant pollen, uninhibited upon selfing, will be obtained, and in frequencies considerably lower than those for haploid uninhibited mutant pollen,

TABLE 11. *Ratios between tetraploid and diploid frequencies of mutant elimination of incompatibility specificities in the pollen under the assumption of different ways of action of the two incompatibility loci.*

In four regards, *a*—*d*, alternatives of two contrasting ways are combined for each locus, + denoting the »positive» way, — denoting the »negative» way. The pairs of ratios given refer to numbers of mutant tetrads and numbers of mutant pollen grains, respectively. For further explanation, see text.

Key to the denotations of *a*—*d* (the »positive» way):

a. Locus capable of mutations resulting in uninhibited pollen at the haploid level.

b. One allele of the locus matched in the style, a sufficient prerequisite for incompatibility of the diploid pollen.

c. Double reduction obtains at the locus.

d. Matched heteroalleles of the locus, effective alone in producing incompatibility of the diploid pollen.

	S Z	S Z	S Z	S Z	S Z	S Z	S Z	S Z
a	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
b	+ +	+ +	+ −	+ +	+ −	+ −	+ +	+ −
c	− +	− +	− +	− +	− +	− −	− +	− −
d	+ +	+ −	+ +	− +	+ −	+ +	− −	+ −
	1	2	3	4	5	6	7	8
Tetrad ratio 4n/2n	1/7 · 0.1	1/7 · 0.1	1 · 0.1	1/7	1 · 0.1	1 · 0.1	1/7	1 · 0.1
Pollen ratio 4n/2n	1/14 · 0.1	1/14 · 0.1	1 · 0.1	1/14	1 · 0.1	1 · 0.1	1/14	1 · 0.1

	S Z	S Z	S Z	S Z	S Z	S Z	S Z	S Z
a	− +	− +	− +	− +	− +	− +	− +	− +
b	+ +	+ +	+ −	+ +	+ −	+ −	+ +	+ −
c	− +	− +	− +	− +	− +	− −	− +	− −
d	+ +	+ −	+ +	− +	+ −	+ +	− −	+ −
	9	10	11	12	13	14	15	16
Tetrad ratio 4n/2n	2/7 · 0.1	2/7 · 0.1	2 · 0.1	2/7	2 · 0.1	2 · 0.1	2/7	2 · 0.1
Pollen ratio 4n/2n	1/7 · 0.1	1/7 · 0.1	2 · 0.1	1/7	2 · 0.1	2 · 0.1	1/7	2 · 0.1

obtained from diploid rye. When calculating the relative frequencies of uninhibited diploid pollen, the following considerations are essential.

(1) For the sake of simplicity, double reduction, when present at the Z locus, is assumed to follow from random chromatid assortment. It should be observed that, in the diploid, a mutated PMC will always give a tetrad with two uninhibited pollen grains. In the tetraploid on random chromatid assortment, 1 mutated PMC out of 7 will give rise to one, and only one, double reduction pollen grain, homoallelic for the mutated factor. Provided that the loci are not able by themselves to produce incompatibility (through matched heteroalleles of the pollen), such a pollen grain will always be uninhibited. It is assumed now that the minimal prerequisite for incompatibility of the diploid pollen is contributed, when a single allele at the Z locus is matched in the style, and that the mutation frequency of the individual factor is unchanged after chromosome doubling. The ratio between »tetraploid» and »diploid» tetrads, containing at least one potentially uninhibited pollen grain, will then be equal to $4\times{}^1/_7 / 2\times 1 = {}^2/_7$ (when only the Z locus is capable of mutations resulting in self-compatibility at the haploid level), and $4\times{}^1/_7 / 4\times 1 = {}^1/_7$ (when both loci are capable of such mutations). Considering the numbers of uninhibited pollen grains, the corresponding ratios will be $4\times{}^1/_{28} / 2\times{}^2/_4 = {}^1/_7$, and $4\times{}^1/_{28} / 4\times{}^2/_4 = {}^1/_{14}$, respectively. These distinctions might be of significance, if relatively more uninhibited pollen grains were to be needed in the tetraploid for one fertilization to be performed.

(2) When it is a prerequisite for incompatibility of the diploid pollen that both alleles of the Z locus are matched in the style, even a single mutated factor might render the diploid pollen uninhibited.

(3) Whether occurring double or single, however, the mutant factors must be presumed to react in a »recessive» way to matched heteroalleles of the diploid pollen, when the latter are able by themselves to produce incompatibility. It is evident that only the S locus will in that may be able to reduce the frequency of uninhibited pollen due to mutation at the Z locus, as matched heteroalleles and mutated alleles will exclude one another at the Z locus.

(4) The degree to which the S locus will thus influence the frequency of mutant uninhibited pollen will depend on the frequency of heteroallelic pollen segregating at this locus in the population. The tetraploid strain being derived from seven diploid plants (MÜNTZING, 1951), the S locus can be represented here by maximally 14 different alleles. Assuming genotypic equilibrium in the tetraploid strain, and its alleles at

the S locus being equally frequent, the frequencies of the various categories of S genotypes — monoallelic up to tetraallelic — possible in the population at various numbers of alleles, have been calculated from HALDANE's formulae for gametic ratios (HALDANE, 1930) and for numbers of genotypes possible at one locus at varying numbers of alleles (HALDANE, 1948). The calculated frequencies are given in Table 12. Admittedly, no real genotypic equilibrium is probable at the artificial selection applied to the tetraploid strain, but for the present discussion this error has not been considered significant. It is evident that, double reduction being excluded, all genotypes except the tetraallelic ones will give rise to homoallelic pollen, in proportions that vary with the number of different alleles in the genotype. The pooled proportion of homoallelic pollen in the population will vary with the number of alleles at the S locus, present in the population. As can be seen from the last column of Table 12, the proportion of homoallelic pollen can be fairly safely approximated to 10 %, a very low number of alleles in the tetraploid population being most improbable (*cf.* LUNDQVIST, 1956). The frequency of uninhibited diploid pollen due to mutation at the Z locus would thus far become reduced by about 90 %, if heteroalleles at the S locus were able alone to produce incompatibility.

Returning now to Table 11, it will be found that the calculated relative mutant frequencies of the tetraploids vary between 0.7 % and 28.6 %. However, these values taken separately will evidently give no reliable information as to which of the 16 combination patterns is the most probable, several drawbacks being inherent to the analysis. The assumption of a real genotypic equilibrium is somewhat artificial. The frequency of double reduction was calculated from random chromatid assortment, but might quite as well be considerably lower. Moreover, if relatively more uninhibited pollen grains are needed in the tetraploid to effect one fertilization, a considerable under-estimation of the frequency of pollen uninhibited upon selfing may result in the tetraploid (*cf.* ATWOOD and BREWBAKER, 1953; BREWBAKER, 1955).

The 16 combination patterns may, thus, be considered as follows.

(1) Biologically, it seems equally possible that both loci or only the Z locus are capable of mutations, resulting in uninhibited pollen at the haploid level. Special experiments are carried on to see if self-compatibility factors are found at both loci.

(2) It is a known fact that for the S locus the prerequisite for incompatibility of the diploid pollen may be already contributed by a single

matched allele. Biologically, it cannot be judged how the Z locus will behave in this respect.

(3) The occurrence of double reduction is completely due to the frequency of crossing-over in the intercalary region between the incompatibility locus and the centromere, combined with the frequency of quadrivalents formed for the chromosome in question. MÜNTZING (1951) reported quadrivalents to be frequent in the tetraploid strain in question.

(4) As regards the question whether matched heteroalleles of the diploid pollen are capable by themselves of producing incompatibility, the following considerations are important. It is highly probable that incompatibility in rye results from a complementary co-operation between unidentical factors. The pollen control being exerted gametophytically, this co-operation has been evolved at the haploid level, and thus between factors belonging to different loci. Considering the highly specific character of the incompatibility reaction (LEWIS, 1949), this evolution of the factorial co-operation must be well balanced, and a specialization of the two loci, involved together in the incompatibility reaction, is conceivable. Thus, it does not seem very probable that co-operation between alleles belonging to the same locus, theoretically possible in the diploid pollen, will be capable of being well balanced in the same way as evolved for the factorial co-operation between loci. It seems obvious that the factorial co-operation between loci must be at least as effective in evoking inhibition of pollen tube growth as will an occasional allelic co-operation within loci. From these considerations it is improbable that two matched factors will be more effective, when they belong to the same locus (heteroalleles), and combinations Nos. 3, 5, 6, 8, 11, 13, 14, and 16 are thus excluded. Combinations Nos. 2, 4, 10, and 12 would appear more probable, had matched heteroalleles been effective at both loci. Of the remaining combinations, Nos. 1 and 9 assume general effectiveness of matched heteroalleles and are judged less probable from the foregoing considerations. Moreover, the values for relative mutant frequencies, obtained here, are quite considerably below the observed ratio. Combinations Nos. 7 and 15 would thus far remain probable. Special experiments are designed, however, to get critical information on the effects of matched heteroalleles.

On the given presumptions, the control of incompatibilities in tetraploid Steel-rye would appear to be exerted as follows. The single locus is not able alone to produce inhibition of pollen tube growth. *The pollen specificities result from co-operation between the loci within pairs of*

factors. One such factorial pair of the pollen matched in the style is sufficient for incompatibility.

If the control of the tetraploid incompatibilities really follows the suggested scheme, the way of calculating Table 12 is not absolutely correct. One factorial pair of the pollen, matched in the style, being sufficient for incompatibility, the genotype categories double monoallelic, monoallelic+unbalanced diallelic, and double unbalanced diallelic will not be obtained in the offspring. The frequency of monoallelics and unbalanced diallelics thus being reduced, the genotypic equilibrium of the population will be changed to a higher frequency of heteroallelic pollen. The lower the number of incompatibility alleles in the population, the greater this change of equilibrium will be. Assuming a population with only two alleles at each of the loci, and considering the two alleles of the S locus only, the pollen genotypes $S_{1.1}$ and $S_{1.2}$ will approach an equilibrium ratio of about $^1/_3$. With an increasing number of incompatibility alleles in the population, this equilibrium ratio will approach the value $^1/_2$, underlying the calculations of Table 12; and it has been considered that, at least for the higher numbers of alleles, the error introduced in Table 12 may not be considerable, and that the conclusions above may still be valid.

No oppositional allelic data being obtained, the presence or absence of competitive allelic interaction within the loci, resulting in dominance or mutual weakening in the diploid pollen, cannot be established with certainty. Dominance allelic interaction within the locus may of course result in the observed inhibition of diploid pollen with less than all four factors matched in the style, but this will not essentially influence the validity of the previous discussion and conclusions.

As to allelic interaction resulting in mutual weakening, 14 different alleles at each locus are maximally possible in the tetraploid population (the latter being derived from 7 diploid plants), and strongly competitive allelic combinations ought to have had a fair chance to be tried out; but self-compatible individuals were not observed in the highbred tetraploid strain of Steel-rye. This fact does not definitely rule out allelic mutual weakening in the diploid pollen of rye, for it has been found that allelic interaction, resulting in mutual weakening and self-compatibility, may be infrequent in new artificial polyploids of *Trifolium* (BREWBAKER, 1953, 1954; PANDEY, 1956); and in *Oenothera organensis* competitive allelic interaction was not strong enough to give any seed after selfing (LEWIS, 1947).

A slight increase of seed-setting upon selfing was observed in the

TABLE 12. *Proportions of the five plant genotype categories in an autotetraploid population at genotypic equilibrium, one locus, G, being considered, with n alleles, all equally frequent; and the proportions of homoallelic pollen formed from these populations upon chromosome assortment.*

Gametic ratio for individual homoallelic and heteroallelic genotypes, *e. g.*, $G_{1.1}$ and $G_{1.2}$, at chromosome assortment, is $1\,G_{1.1}/2\,G_{1.2}$ (HALDANE, 1930). Numbers of plant genotypes possible, according to HALDANE (1948).

		Plant genotype categories					Total	Homoallelic pollen in %
		mono-all.	diall. unbal.	diall. bal.	triall.	tetra-all.		
a	Relative frequency of the individual plant genotype [Ways of combining the gametes · (frequency of female gamete · frequency of male gamete)]	$1 \cdot 1 = 1$	$2(1 \cdot 2) = 4$	$2(1 \cdot 1) + 2 \cdot 2 = 6$	$2(1 \cdot 2) + 2(2 \cdot 2) = 12$	$6(2 \cdot 2) = 24$		
b	Numbers of genotypes possible	n	$n(n-1)$	$n(n-1)/2$	$n(n-1)(n-2)/2$	$n(n-1)(n-2)(n-3)/24$		
a · b	Proportions of the genotype categories at n different alleles at the locus: 2	2	8	6	0	0	16	50.0
	3	3	24	18	36	0	81	33.3
	4	4	48	36	144	24	256	25.0
	5	5	80	60	360	120	625	20.0
	6	6	120	90	720	360	1296	16.7
	7	7	168	126	1260	840	2401	14.3
	8	8	224	168	2016	1680	4096	12.5
	9	9	288	216	3024	3024	6561	11.1
	10	10	360	270	4320	5040	10000	10.0
	11	11	440	330	5940	7920	14641	9.1
	12	12	528	396	7920	11880	20736	8.3
	13	13	624	468	10296	17160	28561	7.7
	14	14	728	546	13104	24024	38416	7.1
	Proportions of formed pollen being homoallelic	6/6	3/6	2/6	1/6	0/6		

tetraploid strain of Steel-rye, and may result from a direct, weakly competitive, allelic interaction within loci; but other explanations may be possible. In the haploid pollen, there is only one pair of co-operating factors; in the diploid pollen there are four such pairs (between loci), all different from one another in the double heteroallelic pollen, of two kinds in the single heteroallelic pollen, and of one kind in the double homoallelic pollen. A more indirect competition, between unidentical factorial pairs, would thus be conceivable. Finally, other things being equal, an increase of the threshold for the incompatibility reaction in the diploid pollen may effect an impairment of the incompatibility reaction in the tetraploids.

To understand the evolution of the present incompatibility system, it may be important to establish critically the occurrence or absence of allelic interaction within loci in the diploid pollen. The complementary co-operation between loci in the haploid pollen, without observable competitive factorial interaction, could conceivably result secondarily; the two loci primarily being more or less homologous, tendencies to competitive interaction between the loci being selected against, the selection ultimately resulting in complementary co-operation between the loci. Thus far the organism would conceivably be fairly well prepared also to avoid allelic competitive interaction within loci, after chromosomal doubling, in the diploid pollen; the better prepared the more homologous the loci originally were. From the present material it seems most probable that the two incompatibility loci, present in the rye material, do not act identically, but from this conclusion alone no further conclusions concerning their origin can be drawn. It will be necessary to attack the problem on perennial, more favourable materials. Such materials may be found in *Festuca pratensis* (LUNDQVIST, 1955) and *Phalaris coerulescens* (HAYMAN, 1956), where identical incompatibility systems seem to be operating.

V. PRACTICAL IMPLICATIONS

When self-incompatibility is not eliminated upon chromosomal doubling, sterility and reduction of seed-setting due to the lack of compatible pollination, may become a serious problem in an autotetraploid, particularly at a low number of incompatibility alleles in the tetraploid population (LEWIS, 1943, 1954). From the fact that not all factors of the diploid pollen need be matched in the style for the pollen to react incompatibly, this problem will become considerably aggravated in the case of autotetraploid rye.

General cross-compatibility in terms of compatible pollen may be most simply expressed through the proportion of incompatible combinations between the pollen genotypes and style genotypes possible in the population. For the sake of simplicity the whole of the following discussion will start from the assumption of the incompatibility alleles being equally frequent in the population and equally apportioned to the two loci, with n alleles at each, and with random distribution of the possible genotypes in the population. One factorial pair of the diploid pollen matched in the style is assumed to be sufficient to render the pollen incompatible. Thus the three genotype categories, the double monoallelic (*e. g.*, $S_{1.1.1.1}Z_{3.3.3.3}$), the monoallelic+unbalanced diallelic (*e. g.*, $S_{1.1.1.1}Z_{3.3.3.4}$), and the double unbalanced diallelic (*e. g.*, $S_{1.1.1.2}Z_{3.3.3.4}$) will not normally be obtained in the offspring upon cross-pollination. The number of possible pollen genotypes will be $= \left[\frac{n(n+1)}{2}\right]^2$, and the number of possible style genotypes will be $= \left[\frac{(n+3)!}{(n-1)!\,4!}\right]^2 - n^4$, where n^4 is the number of the genotypes eliminated above (*cf.* HALDANE, 1948). The number of possible combinations between pollen genotypes and style genotypes is obtained as the product of these two expressions.

The number of incompatible pollen-style combinations may be obtained as follows. Nine main categories of style genotypes are possible (monoallelic+diallelic, up to tetraallelic+tetraallelic). The numbers of genotypes, by which they will be represented, may be calculated from HALDANE's formulae for numbers of genotypes possible at one locus at varying numbers of alleles (HALDANE, 1948). The numbers of pollen genotypes that they will inhibit may be obtained as pair products from the general expression $\frac{a(2n-a+1)}{2}$, where a denotes the number of different alleles at the locus in the style genotype in question. Thus, the genotype $S_{1.2.3.3}Z_{4.5.6.7}$ (triallelic+tetraallelic) will inhibit $(3n-3)(4n-6)$ pollen genotypes. The total number of incompatible pollen-style combinations may thus be expressed as

$$\frac{n^2(4n^6+12n^7+25n^6+36n^5-542n^4+1176n^3-1143n^2+576n-144)}{144}.$$

The percentage of incompatible pollen-style combinations in tetraploid populations, calculated in that way for $n=2$—$n=7$ and for $n=14$, are given in Table 13, together with the corresponding values for the diploid populations under equal assumptions, as calculated from corres-

ponding expressions (LUNDQVIST, 1954). It can be seen from the table that the proportion of incompatible pollen-style combinations is very high in the tetraploids, and quite considerably higher than in the diploids.

Now the above way of calculating this reduction of general cross-compatibility is not quite correct, no regard being paid to the genotypic equilibrium of the populations. For the diploid populations the correction is easily achieved. The ratio of double heterozygotes to single heterozygotes in the diploid population at equilibrium being $\frac{n-1}{2}$ (LUNDQVIST, 1956), each double heterozygous style genotype inhibiting 4, and the single heterozygous style genotypes inhibiting 2 pollen genotypes, and the number of pollen genotypes being n^2, the proportion of incompatible pollen-style combinations in the population will be $\frac{4}{n^2} \times \frac{n-1}{(n-1)+2} + \frac{2}{n^2} \times \frac{2}{(n-1)+2} = \frac{4}{n(n+1)}$. The values obtained in that way are also given in Table 13. It will be seen that, from the application of the first method, there follows only a slight under-estimation of the proportion of incompatible combinations.

TABLE 13. *Reduction of general cross-compatibility in per cent in an autotetraploid and a corresponding diploid population with n alleles, all equally frequent, at each of two incompatibility loci.*

One factorial pair of the diploid pollen matched in the style is assumed to be sufficient to render the pollen incompatible, when these two factors are located at different loci. For further explanation, see text.

	Per cent incompatible pollen-style combinations at	Number of alleles, n						
		2	3	4	5	6	7	14
Tetraploids	Equal frequency of the possible genotypes	85.2	70.7	59.4	50.3	42.9	37.0	16.1
	Approximated genotypic equilibrium. Chromosome assortment at both loci	96.6	88.2	77.9	67.1	57.4	49.2	19.9
	Appr. gen. equilibr. Random chromatid assortment at one locus	95.5	84.5	72.3	60.7	51.1	43.1	16.8
	Appr. gen. equilibr. Random chromatid assortment at both loci	94.3	80.9	66.9	54.9	46.1	37.8	14.1
Diploids	Equal frequency of the possible genotypes	60.0	29.6	17.9	12.0	8.6	6.5	1.8
	Genotypic equilibrum	66.7	33.3	20.0	13.3	9.5	7.1	1.9

A corresponding under-estimation, but much more serious, is obtained in the tetraploid series. The strict correction is difficult to obtain. The incompatibility elimination of the three above-mentioned oligoallelic style genotypes will change the genotypic equilibrium of the population to a higher frequency of heteroallelic pollen. As may be realized from the admittedly incorrect Table 12, this change of equilibrium will be inversely correlated with the number of different incompatibility alleles in the population. Considering individual genotypes of the three pollen categories, the double homoallelic, the single heteroallelic, and the double heteroallelic ones, their ratio at $n=2$ will be about 1 : 3 : 9, and with an increasing value for n, the ratio will approach the values of 1 : 2 : 4, obtained from Table 12. For the following discussion it has been considered a sufficient approximation to start from the latter equilibrium ratio, when calculating the proportion of incompatible pollen-style combinations; only it must be remembered that the proportions thus obtained constitute minimum values.

(1) The style populations are calculated from Table 12. Separating balanced diallelic genotypes from the unbalanced diallelic ones, and omitting the three incompatibly eliminated genotype categories, the proportions of the various categories of style genotypes are obtained.

(2) For the sake of simplicity, assuming chromosome assortment at both loci, the proportions of the three pollen categories, the double homoallelic, the single heteroallelic, and the double heteroallelic ones, formed from these populations, are easily calculated.

(3) The total number of possible pollen genotypes, $\left[\frac{n^2+n}{2}\right]^2$, are apportioned to the three pollen categories according to the following expressions:

Double homoallelic genotypes $=n^2$
Single heteroallelic genotypes $=n^2(n-1)$
Double heteroallelic genotypes $=\frac{n^2(n-1)^2}{4}$

(4) The style genotype categories may be denoted as S_aZ_b, where a and b denote the numbers of different alleles, represented in the genotype, at the S- and the Z locus, respectively. From the three pollen categories the following numbers of pollen genotypes will thus be inhibited:

ab double homoallelics

$\frac{ab(4n-a-b-2)}{2}$ single heteroallelics

$\frac{ab(2n-b-1)(2n-a-1)}{4}$ double heteroallelics

The relative frequencies with which the different style genotype categories [denoted according to (4)] and the three pollen genotype categories will be represented in the population are thus obtained from (1) and (2), respectively. The ratio of pollen genotypes within each of the three pollen categories that a certain style genotype category will be able to inhibit, will be obtained through $\frac{(4)}{(3)}$. The proportion of incompatible pollen-style combinations in the population will thus be equal to the sum of the products of $(1)\times(2)\times\frac{(4)}{(3)}$ (maximally 27 such products, from 3 pollen, and 9 style genotype categories). It can be seen from Table 13 that, at $n=7$, about 50 % of the combinations will be incompatible in the tetraploid population, and even at $n=14$ the proportion of incompatible combinations is still as high as 20 %, whereas the corresponding value is only 2 % in the diploid population.

Allowance for chromatid assortment will increase the proportion of homoallelic pollen and thus decrease the proportion of incompatible pollen-style combinations in the population. A table corresponding to Table 12 may be constructed, assuming random chromatid assortment, and without correction for elimination of the three above-mentioned oligoallelic style genotypes. The genotypic equilibrium ratio for the individual homoallelic and heteroallelic pollen genotypes, *e. g.*, $Z_{a.a}:Z_{a.A}$, will thereby be $(n+4):8$, the ratio thus being changed in favour of the individual homoallelic pollen genotype with an increasing number of different Z alleles in the population. Assuming random chromatid assortment at one or both of the incompatibility loci, the proportions of incompatible pollen-style combinations are calculated for $n=1$—7, and $n=14$, and are also given in Table 13.

The increase of general cross-compatibility due to double reduction homoallelic pollen does not seem very considerable, but in any case a selective premium is put at such pollen. It is not known whether the high positive correlation between seed-setting and proportion of compatible pollen, demonstrated for autotetraploid *Trifolium repens* (ATWOOD and BREWBAKER, 1953; BREWBAKER, 1955), is also valid for autotetraploid rye. The lower seed-setting in cross-pollinated autotetra-

ploid rye has been shown to a considerable degree to follow from zygotic disturbances during seed development (HÅKANSSON and ELLERSTRÖM, 1950), probably mainly resulting from aneuploidy due to the irregular mode of meiosis with a high frequency of quadrivalents (MÜNTZING, 1951). HÅKANSSON and ELLERSTRÖM (*l. c.*) also found, however, that failure of fertilization of apparently normal embryo sacs was more frequent in the tetraploid strains, and as a possible cause they point out decreased efficiency of the diploid pollen, as made probable for rye by ELLERSTRÖM (cited from MÜNTZING, *l. c.*), and demonstrated for maize by RANDOLPH (1935). MÜNTZING (*l. c.*) pointed out the existence of purely mechanical reasons for the failure of fertilization, the pollen grains of the tetraploid strain being larger and heavier, less easily carried by the wind; and the importance of the density of the stand was indicated, seed-setting actually being lower in less dense stands of an American variety of tetraploid rye. Thus the diploid pollen in rye is obviously functionally handicapped, and a reduction of the amount of available functioning pollen may therefore be much more serious in tetraploid rye.

Under these conditions incompatibility may form one of the most important causes of sterility in an autotetraploid rye population with a narrow genetic basis, particularly at adverse external conditions; and factors bringing about an increase of the proportion of compatible pollen may be selectively favoured. It is an interesting fact, as pointed out by MÜNTZING (*l. c.*), that rigid selection for increased fertility performed during 7 years in the autotetraploid strain of Steel-rye, has obviously had little or no effect on the mode of chromosome pairing at meiosis, the frequency of quadrivalents being as high here as in recent varieties not subject to selection. In view of the above considerations this fact may not be quite unexpected. In any case, it should be observed that if all four factors of the diploid pollen had to be matched in the style for the pollen to be inhibited, the selective premium would be put at heteroallelic pollen instead, and the selection would be against double reduction homoallelic pollen. The breeding for a regular meiosis of the tetraploid would be much easier in this case.

It should, however, be admitted here that the autotetraploid strain of Wasa II, derived from only 3 plants, has a seed-setting not much below that of the strain of Steel-rye derived from 7 plants (MÜNTZING, *l. c.*), and that, notwithstanding its considerably broader genetic basis (SENGBUSCH, 1940), multivalents are common in the highbred tetraploid strain of Petkus rye, though essentially less frequent than in Steel-rye (PLARRE,

1954). It should also be observed that BLEIER (1949) reports a considerable improvement of fertility to be obtained in tetraploid Petkus rye after selection against irregular meiosis; PLARRE (*l. c.*), also on tetraploid Petkus rye, reached the same conclusion.

In any case oppositionally controlled incompatibility will add to the factors giving haploid pollen an advantage over diploid pollen in tetraploid styles; this sensitivity to haploid pollen, characterizing tetraploid rye, was demonstrated by SENGBUSCH (1940, 1941), HÅKANSSON and ELLERSTRÖM (1950), LÖWENSTEIN (1951), MÜNTZING (1951), HAGBERG (1953). It seems reasonable that, with a broader genetic basis of the tetraploid population, seed yield will become less negatively affected from this sensitivity.

Selection for higher yield in tetraploid rye must obviously avoid a reduction of the population pool of incompatibility alleles. Here the increased stability in the tetraploid against loss of alleles by random drift, will be a factor of security.

Under the same assumptions as before, the number of the possible style genotypes at the single locus will be $\frac{(n+3)!}{(n-1)!\,4!}$ (HALDANE, 1948), here denoted as A; with both loci combined, the number will accordingly be denoted as $(A^2 - n^4)$, as demonstrated above. Considering the style genotypes at the single locus, the following numbers of genotypes in the five genotype categories (monoallelics up to tetraallelics) will carry a certain allele, say S_1:

In the monoallelics $= 1$	$(2n-1)$
» » unbalanced diallelics $= 2(n-1)$	
» » balanced diallelics $= (n-1)$	
» » triallelics $= \frac{3(n^2-3n+2)}{2}$	$\frac{n^3+3n^2-10n+6}{6}$
» » tetraallelics $= \frac{n^3-6n^2+11n-6}{6}$	

When combining the two loci, the monoallelics and unbalanced diallelics can only be combined with balanced diallelics, triallelics, and tetraallelics, whereas the latter categories can be combined at will. The allele S_1 will thus be found in $(2n-1)(A-n^2) + \frac{(n^3+3n^2-10n+6)}{6} \times$ $\times A$ genotypes of the $(A^2 - n^4)$ style genotypes, which are totally possible. Corresponding expressions for diploid rye populations are given (LUNDQVIST, 1954). From Table 14 it can be seen that the probability of

finding the individual incompatibility allele will be considerably higher in the tetraploid populations.

Taking into consideration the genotypic equilibrium of the populations (see above), it will be found that an under-estimation of the degree to which an allele will be represented in the population is obtained above. For the diploid populations the correction is easily achieved. The number of gametic genotypes, all equally frequently represented, being n^2, the number of different zygotic genotypes after the incompatibility elimination of double homozygotes will be $(n^4 - n^2)$. The number of gametic genotypes with a certain allele, say S_1, is n, the remaining number of genotypes being $(n^2 - n)$. In the offspring, the proportion of individuals carrying S_1 will thus be

$$\frac{(n^2-n)+2n(n^2-n)}{n^4-n^2}=\frac{2n+1}{n(n+1)}.$$

It may be seen from Table 14 that the under-estimation, resulting from the first method, is quite insignificant. For reasons outlined above, the strict correction is difficult to obtain for the tetraploids. It has, however, been considered here a sufficient approximation to calculate the style populations from the equilibrium underlying Table 12 (see above). For the sake of simplicity, chromosome assortment is assumed at both

TABLE 14. *Probability, expressed in per cent, of finding a certain incompatibility allele in an autotetraploid and a corresponding diploid population with n alleles, all equally frequent, at each of two incompatibility loci.*

One factorial pair of the diploid pollen matched in the style is assumed to be sufficient to render the pollen incompatible, when these two factors are located at different loci. For further explanation, see text.

	Style genotypes containing a certain allele at	Number of alleles, n						
		2	3	4	5	6	7	14
Tetraploids	Equal frequency of the possible style genotypes	88.9	72.9	60.7	52.0	45.7	40.8	24.0
	Approximated genotypic equilibrium. Chromosome assortment at both loci	96.2	82.4	69.2	59.4	52.0	46.1	25.7
Diploids	Equal frequency of the possible style genotypes	80.0	55.6	42.9	35.0	29.6	25.7	13.4
	Genotypic equilibrium	83.3	58.3	45.0	36.7	31.0	26.8	13.8

loci. The numbers of different genotypes in the five style categories at the S locus (monoallelic—tetraallelic) are obtained from HALDANE's formulae (HALDANE, 1948), and the various probabilities of finding a given allele, say S_1, represented in the genotypes of these five genotype categories are easily obtained from the five expressions above. Applying these probabilities to the calculated relative frequencies of these style genotype categories, the occurrence of the given allele in these populations is obtained. As can be seen from Table 14, the stability against loss of alleles is greatly increased in the tetraploids.

SUMMARY

(1) Self- and cross-incompatibilities in autotetraploid rye are studied in the highbred strain of Steel-rye, using seed-setting as the criterion. Self-incompatibility is not absolute in the tetraploid nor in the diploid strain, self-incompatible plants frequently setting some few seeds upon selfing.

(2) No fully self-compatible plant was obtained in the tetraploid élite populations among 974 plants selfed. Also in the S_1 generation the frequency of tetraploid self-compatibles derived from a self-incompatible parent plant is clearly lower than the corresponding frequency in the diploids. Comparing the self-incompatible plants, however, seed-setting upon selfing is slightly but significantly higher in the tetraploid élite populations, and the variability in seed-setting between different years is much higher in the tetraploids.

(3) From intercrosses between chromosome doubled siblings, obtained from the diploid sib combination ho het $\times$ het het, evidence is obtained that two homologous alleles (homoalleles) of the pollen, matched in the style, are not able alone to render the diploid pollen incompatible.

(4) Upon intercrosses in four tetraploid S_1 families, obtained from selfed élite population plants of unknown genotype, several reaction patterns, primarily the ununiform reaction pattern within groups of reciprocally incompatible plants, indicate that, at least for one locus, not both alleles of the pollen must be matched in the style for the pollen to react incompatibly, and that double reduction does not obtain for this locus.

(5) The combined evidence points to the view that in the diploid pollen the single locus is not able alone to produce inhibition of pollen tube growth. The pollen specificities result from co-operation between

the loci within pairs of factors. One such factorial pair of the pollen matched in the style, is sufficient for incompatibility.

(6) No oppositional allelic data being obtained, the presence or absence of competitive allelic interaction within the loci, resulting in dominance or mutual weakening in the diploid pollen, cannot be established with certainty.

(7) Formulae for calculating general cross-compatibility and stability against loss of alleles due to random drift are given for the tetraploid population under the conditions outlined in (5) above. Lack of compatible pollination in the tetraploid rye may be a problem more serious than hitherto expected, and breeding for a regular meiosis in the tetraploid may be difficult. The stability against loss of alleles is greatly increased at the tetraploid level.

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PRODUCTION OF POLYPLOIDS AND ANEUPLOIDS OF PHALARIS BY MEANS OF NITROUS OXIDE

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A few years ago it was demonstrated by the present writer (ÖSTERGREN, 1954) in experiments with *Crepis capillaris* that nitrous oxide could be used successfully for the production of polyploid plants. The flowers were pollinated and when the first mitoses passed in the resulting zygotes the plants were treated with nitrous oxide of 10 atm. pressure for 4 hours. Some success was also obtained by treating for 6 hours at the time when the second zygotic division was taking place.

It was considered of interest to test the method also on some other species and for this reason efforts were made to find out when the first zygotic divisions take place. The results, however, were rather disappointing. In the materials studied (*Nigella*, *Papaver*, *Dipcadi*) it was found very difficult to determine the time in question. The zygote appears to divide only several days after pollination and not simultaneously in different ovules. For this reason no treatments were made with these species.

It is, however, obvious that it should also be possible to get polyploids when treatments are applied to other stages than the first zygotic division. When a later mitosis is treated the embryo should become a chimaera. In some cases the resulting polyploid cells may be located in such a position in the embryo that they will give rise to a meristematic region and the resulting seedling may become a polyploid. Furthermore, in many species there are inflorescences in which the flowers represent a practically continuous series of various developmental stages. When a treatment is applied to a material of this kind there should obviously be a good chance that some of the zygotes are in a stage sensitive to the treatment. For these reasons there should be some chance of getting a successful result even when a treatment is applied rather at random without any knowledge of when the first mitosis is taking place.

TABLE 1. *Progenies of plants of Phalaris canariensis (2n=12) treated with N_2O of 10 atm. pressure.*

Progeny no.	Total number of plants	Plants selected for chromosome study	Heteroploids	Duration of treatment, hours
1	13	1	1	7
2	6	3	1	$8\,^1/_2$
3	—	—	—	13—14
4	6	2	1	6
5	5	2	1	$8\,^1/_2$
6	2	—	—	10
Total	32	8	4	

Two of the heteroploids were tetraploids ($2n=24$) and two were aneuploids having $2n=23$ and $2n=21$, respectively.

Some experiments in which the treatment was applied in this more random way were performed on *Phalaris canariensis* and *Ph. paradoxa*. These materials seem to be self-fertile and are probably to a large extent self-fertilizing. Each panicle contains a series of different stages, the oldest flowers being at the top and the youngest at the base. The treatment with nitrous oxide was applied when the youngest flowers had just completed their flowering and the older flowers were a few days after flowering. In all cases nitrous oxide of 10 atm. pressure was applied. The treatment was performed in the same way as in the *Crepis* experiments (ÖSTERGREN, 1954). The plants treated had one or two panicles each at the stage mentioned above. The result of the experiments can be seen in Tables 1 and 2. Some progeny plants of a more or less polyploid appearance were selected for chromosome study and some of these were found to be heteroploid.

The results obtained show that a satisfactory yield of polyploids can be obtained in these materials by means of the method used. In addition to 4 tetraploids, 2 aneuploids were obtained. These probably result from an incomplete spindle-inactivation of the type suggested to explain the corresponding phenomenon in my *Crepis* experiment (ÖSTERGREN, 1954).

I have made similar experiments on some other species, but as no progenies have as yet been investigated, I have no information concerning the results.

A very satisfactory result was obtained by NYGREN (1955) who induced polyploidy in *Melandrium* (*album* and related materials) by

TABLE 2. *Progenies of plants of Phalaris paradoxa (2n=14) treated with N_2O of 10 atm. pressure.*

Progeny no.	Total number of plants	Plants selected for chromosome study	Heteroploids	Duration of treatment, hours
7	7	—	—	5
8	13	3	1	4
9	18	1	—	4 1/2
10	14	7	1	12
11	6	—	—	6
12	6	2	—	6
Total	64	13	2	

The two heteroploids were tetraploids ($2n=28$).

means of treatment with nitrous oxide. Thus, in his treatments with 5 atmospheres he got 86,1 % polyploids. As he studied a great number of plants, there can be no doubt that the nitrous oxide method is very efficient in *Melandrium*. For comparison one may cite the results of TAKENAKA (1954) who produced polyploids of *Melandrium* by means of colchicine treatment of young plants. When using the optimal conditions of concentration and duration of treatment, he produced 27 tetraploids out of a total of 56 plants (48,2 %). WESTERGAARD (1940), using the method of treating the dividing zygote with a heat shock, obtained about 4 % tetraploids in *Melandrium* from his best seed capsule (2 plants out of 48).

A surprising feature in NYGREN's results is the high frequency of triploids that he obtained and the occurrence of a few hexaploids. It may be somewhat doubtful whether these triploids could all be produced by multipolar mitoses in the manner suggested by me for the triploids of *Crepis capillaris*. If this mechanism had operated, a higher frequency of aneuploids would have been expected in *Melandrium*. For this reason some other interpretation might be looked for as an alternative explanation.

One way in which triploids might be considered to originate would be through an influence of the nitrous oxide on the second pollen mitosis, in which case a single diploid sperm nucleus would be obtained instead of two haploid ones. If this diploid sperm nucleus fertilizes the egg cell and the central nucleus of the embryo sac is fertilized by another pollen tube, a seed with a triploid embryo would result. This interpretation would be applicable to the *Melandrium* case only if the

second pollen mitosis takes place in the pollen tube. As, however, the ripe pollen grains of *Melandrium* already contain two sperm nuclei (SCHNARF, 1931), this hypothesis is ruled out. For the same reason a mechanism of this kind cannot have contributed to produce triploids in the *Crepis* experiment. In *Crepis*, too, the ripe pollen contains two sperm nuclei (SCHNARF, 1931; HEITZ, 1950).

Another and more probable hypothesis to explain the relatively high frequency of triploids in the *Melandrium* experiment is to suppose that there has been polysperm fertilizations. In normal cases there must operate a special mechanism which prevents the egg cell from being fertilized by more than a single sperm. The results of the *Melandrium* experiment may suggest that the nitrous oxide treatment had decreased the efficiency of the normal barrier against polyspermy. It is obvious that polyspermy could lead to the production of triploids as well as tetraploids in this experiment. In order to bring about a fertilization of both the egg cell and the central nucleus of the embryo sac in such cases, more than one pollen tube must penetrate each embryo sac.

The hexaploids obtained by NYGREN might have originated in the following way. First a triploid zygote is obtained through polyspermy. When this divides in its first mitosis, the spindle is inactivated by the nitrous oxide and a hexaploid cell is obtained through c-mitosis. The few aneuploids of the *Melandrium* experiment probably result from the multipolar spindle mechanism suggested by me for *Crepis*. Apart from polyspermy the tetraploids of this experiment may originate from spindle inactivation and c-mitosis at the first zygotic division.

Finally it may be mentioned that it is easy to produce polyploids in *Phalaris canariensis* also by means of colchicine, a fact reported by BREMER-REINDERS and BREMER (1952). We have been able to confirm that observation. Thus, I am not going to state that nitrous oxide is superior to colchicine in this material. On the contrary, it appears that colchicine may be better, in so far as the treatment itself is more easily performed. A large material of colchicine-treated seedlings is easily obtained.

The main importance of the nitrous oxide method would seem to be that it gives us one more method of chromosome doubling to choose from. Different materials react differently towards different methods and for cases in which the colchicine method does not work well it may be of value to have alternative methods to test. It is also possible that nitrous oxide treatment may be a suitable method for the produc-

tion of triploids and aneuploids in materials where other methods are not easily applicable, *e. g.* in cases where diploid and tetraploid races cannot be crossed.

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SUMMARY

Treatment of panicles of *Phalaris canariensis* and *Ph. paradoxa* with nitrous oxide of 10 atmospheres pressure for 4 to 12 hours a short time after flowering induces polyploidy and aneuploidy in some plants of the progeny. A new hypothesis is suggested to explain the relatively high frequency of triploids in NYGREN's (1955) *Melandrium* experiment with nitrous oxide.

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THE CHROMOSOME NUMBER OF HONCKENYA PEPLOIDES (L.) EHRH., WITH A NOTE ON ITS MODE OF SEX DETERMINIATION

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INTRODUCTION

THE chromosome number of *Honckenya peploides* (L.) EHRH. (Caryophyllaceae) was first counted by ROHWEDER (1939), who found two different numbers. A sample from Schönhagener at the Baltic Sea had $n=24$, whereas a second sample from St. Peter at the North Sea coast, was counted to $n=32$. *Honckenya peploides* is sometimes quoted in the literature as a good sample of a species showing »intraspecific polyploidy» with the different polyplotypes having different geographical distribution.

In 1950 FLOVIK counted *Honckenya peploides* from Spitzbergen and Tromsö (Arctic Norway) and found the somatic number to be $2n=66$. Since ROHWEDER's counts indicated 8 as the basic number in *Honckenya*, 66 would be an aneuploid number, for which reason FLOVIK points to the possibility, that the arctic type may have an apomictic reproduction.

Part of the arctic *Honckenya peploides* complex is referred to var. *diffusa* HORNEM. (HORNEMANN 1821: 501). In 1950, A. LÖVE proposed to raise this variety to specific rank: *Honckenya diffusa* (HORNEM.) A. LÖVE. The essential argument for this proposal was FLOVIK's count of $2n=66$ for the arctic type, since this number placed it rather remote from the European plants with $2n=48$ and $2n=64$.

Some years ago, C. A. JÖRGENSEN, TH. SÖRENSEN and M. WESTERGAARD collected material from a number of Danish localities in order to check the chromosome number counted by FLOVIK and ROHWEDER and compare the plants with Greenlandic material. Preliminary investigations carried out in collaboration with P. JACOBSEN failed to show

the variation in chromosome number reported by ROHWEDER, but no definite chromosome number could be ascertained. On their request, Dr. A. ROHWEDER, Kiel, kindly provided new material from his original »48-chromosome» locality at Schönhagener. The whole material was handed over to the present author for a more thorough investigation, the results of which is given in the present paper.

CYTOLOGICAL TECHNIQUE

Plants were raised from seeds and grown in pots out of doors in the Botanical Garden of the University of Copenhagen. In some cases plants were transplanted directly from natural localities and kept in cultivation in the garden. Most of the arctic plants grow poorly and were difficult to keep alive.

Root-tips were fixed in JACOBSEN's fixative (70 per cent alcohol and 1 per cent lactic acid, JACOBSEN, 1954), after 3—4 hours pretreatment with 0.001 mol 8-hydroxyquinoline at 12—14° C (LEVAN and TJIO, 1950). The root-tips were kept at room temperature during the first hour of the pretreatment. The fixative was kept at - 5° C and the root-tips were fixed overnight or longer (but not more than a week) in the cold fixative. The root-tips were transferred directly to 1N HCl and hydrolysed for 8—10 minutes at 60° C. After being rinsed twice in distilled water, they were stained in Feulgen for 1—6 hours, transferred to a drop of 20 per cent acetic acid on the slide, and squashed under the cover-slip. In some cases flattening of the cells was difficult, but this was overcome by treatment with pectinase (SETTERFIELD *et al.*, 1954). The slides were made permanent by the freezing-method and mounted in canada-balsam.

For study of meiosis in PMC's, male flowers were fixed in nature in the modified Carnoy-fixative, described by HYDE and GARDELLA (1953). A stock solution is made by dissolving 400 mgs $Fe(0H)_3$ in 100 ml propionic acid. The fixative is made from three parts of 95 per cent alcohol and one part of the stock solution. The flower buds are put under a suction pump, and fixed at least overnight at - 5° C. The anthers are dissected out, squashed in a drop of aceto-carmine, and handled according to the method of LIMA-DE-FARIA (1948).

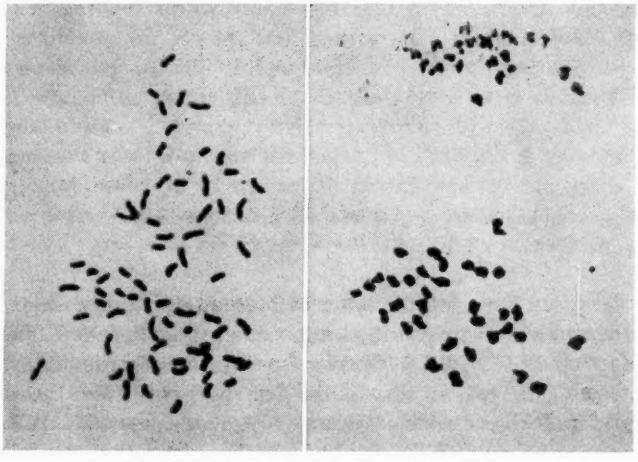
RESULTS

Table 1 summarises the results. The somatic chromosome number of $2n=68$ could be counted with full certainty in the Arctic as well as

TABLE 1. *Chromosome counts.*

Plant no.	Locality	Collector	Sex	Chromosome number
	Denmark			
24/1	Løkken, North Sea	P. JACOBSEN	♀	2n = 68
156	Saltuna, (Bornholm) Baltic Sea	»	♂	2n = 68
	Dragør, Øresund	H. MALLING	♂	n = 34
	Germany			
115	Baltic Sea	H. ROHWEDER	♂	2n = 68
116	» »	»	♂	2n = 68
123	Schönhagener, Baltic Sea[1]	»	♂	2n = 68
	N. America			
109/8	Sitka, Alaska	L. CHRISTENSEN	♂	2n = 68
171	Disko, Greenland	U. RÖEN	?	2n = 68
139	Montreal (var. *robusta* Fern.)	Bot. Garden	♂	2n = 68

[1] Collected 10 meter from the original locality of the »48-chromosome» type, according to a letter from Dr. H. ROHWEDER.



a b

Fig. 1 a. Microphotos of Feulgen stained root-tips squash-preparations of *Honckenya peploides*. Plant no. 115, Baltic Sea; $2n=68$. — Fig. 1 b. Second metaphase, stained with aceto-carmine after pretreatment with iron-proprionic acid; $n=34$. Plants from a natural population near Copenhagen. ×1600.

Fig. 2. Camera-lucida drawing of root-tip squash-preparation. Plant no. 24/1. North Sea; $2n=68$.

in the European material, the latter including the plants collected from Dr. ROHWEDER's original »48-chromosome» type locality (plant no. 123; plants no. 115 and 116 are also from seeds provided by Dr. ROHWEDER, collected at the Baltic Sea, but not from the original locality). Fig. 1 shows microphotos and Fig. 2 a camera-lucida drawing of one of the best plates. It should be remembered that the cells were pretreated with 8-hydroxyquinoline, whereby the chromosomes have become very much shortened. Even so there is some variation in the size of the chromosomes, and one pair carries a very conspicuous satellite.

Meiosis was studied in male plants from Dragør near Copenhagen. The metaphase pairing is regular in most cells, but the chromosome counts were best made on cells in the second metaphase. In the best plates, the haploid number of $n=34$ could be counted to full certainty.

It should be mentioned that in addition to the plants listed in Table 1 which were counted to full certainty, several other plants were studied in which the chromosome number could not be exactly determined. However, all these plants certainly had a somatic number higher than 60, and in no case a chromosome number as low as $2n=48$ (the lowest number reported by ROHWEDER) was found.

DISCUSSION

The conclusion of this investigation must be, that *Honckenya peploides* is monotypic with respect to chromosome number and that the true number is $2n=68$. It is not difficult to understand how this number has been miscounted to $2n=64$ and 66, bearing in mind the difficulty of ascertaining such a high number accurately and also remembering that the earlier counts were not done on squash preparations after pretreatment with 8-hydroxyquinoline. It is much more difficult to explain ROHWEDER's count of $n=24$ which can certainly not be mistaken for $n=34$. Dr. ROHWEDER's fixation could perhaps have been mixed up with flower buds from a different species or the cells on which the count was made may have been touched by the microtome knife.

The present results revoke the basis for FLOVIK's suggestion of an apomictic reproduction of the arctic *Honckenya*, a suggestion which is not supported by any other evidence. They also invalidate the basis for A. LÖVE's proposal of rising the arctic *diffusa* variety to specific rank.

The taxonomical status of the genus *Honckenya* has been much discussed. LINNÉ included it in the genus *Arenaria* from which it was transferred to its own genus by EHRHART (1788). Recently HYLANDER (1941, 1945) has proposed to include the species in the genus *Minuartia* (LOEFL.). Other synonyms are listed by HULTÉN (1944). The present cytological evidence shows that the basic number is 17, whereas the genus *Arenaria* includes species with the basic numbers 10 and 11, and *Minuartia* has the basic number of 13. These fact certainly support the taxonomists who maintain it in a genus of its own.

The basic number of 17 is a very high number which in Caryo-

phyllaceae is found only in the genus *Gypsophila*. It seems very likely that the species is a »secondary polyploid» in DARLINGTON's sense (cf. DARLINGTON and MOFFETT, 1930). As ROHWEDER has pointed out, it is very likely that *Honckenya*, *Cerastium*, and *Sagina* have common ancestors.

The basic number of x=6 and 11 is found in *Sagina* whereas many *Cerastium* species have x=9. These numerical facts may perhaps some day give the clue to our understanding of the evolution of genus *Honckenya*.

A NOTE ON THE MODE OF SEX-DETERMINATION IN HONCKENYA PEPLOIDES

Honckenya peploides is a sub-dioecious species, and the flowers of the two sexes are rather conspicuously different (cf. WARMING, 1877: 127, Fig. 13). The female flowers have very reduced petals, abortive staminodie-like anthers but a normally developed gynoecium. Preliminary investigations indicate that the anthers degenerate before meiosis in the PMC's takes place. The male flowers have much larger, white petals, normally developed anthers and a very reduced gynoecium with three small pistils with very inconspicuous papillae. The embryo-sac formation has not been studied, and we do not know whether the gynoecium degenerates before embryo-sac formation takes place or after the EMC has gone through meiosis; the latter situation is found in *Fragaria* (STAUDT, 1952).

Both male and female plants spread effectively by means of runners and may form very large clones. After flowering in June, the female clones have in July and August plenty of capsules with many seeds, whereas most male clones are seed-sterile. Some male clones may be found, however, which carry a few capsules with normal seeds. It is always the first flower in the cyme which produces such capsules, a phenomenon also found in other dioecious or sub-dioecious species of Caryophyllaceae (*Melandrium*, *Silene*).

Seeds have been germinated from open-pollinated female clones in nature. These seeds gave rise to 79 ♀♀ and 99 ♂♂ (55 per cent ♂♂). A ratio not deviating significantly from the expected 1:1 ratio ($\chi^2 = 3.036$. $P > 5\ \%$), although there is a slight preponderence of male plants. In most dioecious plants, a preponderence of females is found in nature (CORRENS, 1928), but an excess of males is also reported for *Silene Otites* (CORRENS, 1928, p. 99) and *Valeriana dioica* (MOEWUS, 1950).

Seeds were also germinated from a few capsules collected in nature on male clones. They gave rise to 20 plants, 2 of which were females and 18 males. This ratio (2: 18) fits a 1: 3 distribution. A binomial test gives $P \geqslant 16\ \%$ (a similar test for a 1: 2 ratio gives $P \geqslant 2\ \%$).

Such 1: 3 ratios have been found in *Mercurialis* (GABE, 1939; KUHN, 1939), *Thalictrum* (KUHN, 1939) and *Asparagus* (RICK and HANNA, 1943) among the offspring of sub-dioecious male plants. Such ratios are explained by assuming male heterogamy, the male plant being of the constitution XY. Such plants with occasional female flowers when selfed should segregate XX;XY and YY types in the proportion 1: 2: 1. If the YY-plants are viable males, the sex ratio will be 1: 3 (see also WESTERGAARD, 1957). The above data, although preliminary, would then suggest, that the male sex in *Honckenya* is the heterogametic one, and that the YY-type is viable. However, the final proof for such an assumption can only be made by crossing the postulated YY males to XX females. Such a cross should give rise to unisexual families of male plants. This critical cross has not yet been made in *Honckenya*. For this reason our interpretation can not be considered as finally proved.

SUMMARY

The chromosome number of 9 samples of *Honckenya peploides* from Denmark, Germany, Greenland, and Alaska have been counted. All the plants have the somatic number $2n=68$, in contradistinction to earlier counts of $2n=48$, 64 and 66 of this species. The number $2n=68$ is considered to be the only one present in the species. Preliminary investigations of the sex-determining mechanism of *Honckenya*, which is a sub-dioecious species, suggest that the male sex is heterogametic.

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SEGREGATION RATIOS IN PROGENIES OF HYBRIDS BETWEEN NATURAL AND SYNTHESIZED PHLEUM PRATENSE

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THE hexaploid *Phleum pratense* has been found to give an autohexaploid mode of segregation for the investigated genes of albino seedlings (NORDENSKIÖLD 1953, 1954). The present study has been made in order to study the segregation of the chlorophyll-deficient seedlings in hybrid material of a cross between natural and synthesized *P. pratense*.

THE PARENTAL PLANTS OF THE CROSS

The plants of natural *P. pratense*, which has been selected for the cross, belongs to the second isolated generation of a commercial strain of timothy, Svalöf's Omnia. The original Omnia plant (No. 4) has given a segregation ratio of 5634 green : 22 white. At the cytological study of meiosis, bivalents have almost exclusively been found. Only in a few per cent of the studied cells could a quadrivalent be observed and never more than one in a cell (NORDENSKIÖLD 1953).

The inbred plant of *P. pratense*, used as the mother in the cross, is a highly self-sterile individual. It has a simplex constitution for the segregation of green and white seedlings, the ratio obtained from the plant after isolation being 103 : 31. Such a ratio of about 3 : 1 reveals a simplex constitution of at least one locus of the plant, but it does not exclude the possibility of several segregating loci for albino genes. The increase of white seedlings in the progeny of a hexaploid simplex plant segregating for several loci compared with the one segregating for only one locus will hardly be recognized by the obtained ratios. If the plant is segregating in two independent loci, the loci being simplex-simplex, simplex-duplex, or simplex-triplex, the expected ratios are 1.28 : 1,

2.57 : 1, or 2.97 : 1, respectively, if it is segregating as a simplex in only one locus, the ratio is 3 : 1 (NORDENSKIÖLD 1953).

The natural hexaploid *P. pratense* ($2n=42$) is closely related to the wild, diploid species of *P. nodosum* ($2n=14$). By the aid of colchicine treatment a tetraploid strain of *P. nodosum* has been produced. This tetraploid strain has later on been used for a repeated colchicine treatment. From the crosses between the different polyploids obtained by these treatments two hexaploid plants of *P. nodosum* have been produced. These hexaploids have been regarded as a synthesized *P. pratense* on account of their close morphological and cytological similarity to the natural *P. pratense*. The cytological investigation of these two artificial hexaploids, has, however, revealed the fact that their chromosome numbers are supernumerary, *i. e.* $2n=44$ and $2n=45$. During meiosis of the hexaploid *P. nodosum* the extra chromosomes appear as univalents. The other chromosomes form mainly bivalents and only in about 20 per cent of the investigated cells has a quadrivalent been observed (NORDENSKIÖLD 1949).

Regarding genes for chlorophyll deficiencies the synthesized *P. pratense* is supposed to contain very few of these. The tetraploid strain of *P. nodosum* — from which it originates — has not yielded any such seedlings, in spite of the fact that it has been produced from only three diploid plants. Thus, the production of a hexaploid plant from this tetraploid strain, with an originally triplex constitution for a chlorophyll deficiency, must be very unlikely.

CALCULATIONS OF POSSIBLE SEGREGATION RATIOS OF THE HYBRIDS

A natural hexaploid *P. pratense*, segregating in a simplex ratio for green and white seedlings, has been crossed with the synthesized *P. pratense*, the natural one being used as the mother. The synthesized *P. pratense* has been presupposed to contain very few genes for chlorophyll-deficient seedlings and to have no such genes in common with the ones segregating in the plant of natural *P. pratense* under investigation.

All the gametes of a hexaploid simplex individual will contain two or three recessive alleles of the segregating locus (Schedule 1). Thus, the F_1 plants, must obtain two or three recessive alleles of the locus in question. If it is postulated that the synthesized *P. pratense* does not have any recessive alleles for albino seedlings in common with the ones segregating in the mother plant, the first mentioned plant must be of a

SCHEDULE 1. *Calculations of the expected ratios of the cross, when one locus of the mother plant is postulated to segregate in simplex.*

Parents	Aa^5 (simplex) × A^6 (hexaplex)	
Female gametes	Aa^2	a^3
F_1 plants	$A^4\ a^2$	$A^3\ a^3$
Segregation of F_1	non-segreg.	399: 1
Segreg. between F_1	1	1

hexaplex constitution at its corresponding locus. On account of this, all F_1 plants must aquire a triplex constitution, segregating in 399 : 1, or a tetraplex one, with a non-segregating progeny. The plants of these two constitutions will occur in equal numbers (Schedule 1).

The simplex plant used in the cross can, however, be segregating for albino genes in more than one locus. If this were the case, there might occur F_1 plants segregating as triplex for two independent loci with a ratio of 199 : 1 (NORDENSKIÖLD 1953) in addition to the simple triplex plants, giving 399 : 1. The expected ratios of the cross if the mother plant is postulated to be of the simplex-simplex, the simplex-duplex, or the simplex-triplex constitutions, are given in Schedules 2—4.

OBSERVED SEGREGATION RATIOS OF GREEN AND WHITE SEEDLINGS

For practical reasons, the cross between the self-sterile natural *P. pratense* and the synthesized *P. pratense* has been made without any emasculation of the mother plant. All the 166 plants obtained from the cross have been green. If all seedlings harvested from the cross are

SCHEDULE 2. *Calculations of the expected ratios of the cross, when two independent loci of the mother plant are postulated to segregate in simplex.*

Parents	$Aa^5\ Bb^5$ (simplex-simplex) × $A^6\ B^6$ (hexaplex)			
Female gametes	$Aa^2\ Bb^2$	$Aa^2\ b^3$	$a^3\ Bb^2$	$a^3\ b^3$
F_1 plants	$A^4\ a^2\ B^4\ b^2$	$A^4\ a^2\ B^3\ b^3$	$A^3\ a^3\ B^4\ b^2$	$A^3\ a^3\ B^3\ b^3$
Segregation of F_1	non-segreg.	399: 1	399: 1	199: 1
Segreg. between F_1	1	2		1

SCHEDULE 3. *Calculations of the expected ratios of the cross, when two independent loci of the mother plant are postulated to segregate in simplex and duplex.*

Parents	$Aa^5 B^2b^4$ (simplex-duplex) × $A^6 B^6$ (hexaplex)					
Female gametes	$Aa^2 B^2b$	$3\ Aa^2 Bb^2$	$Aa^2 b^3$	$a^3 B^2b$	$3\ a^3 Bb^2$	$a^3 b^3$
F_1 plants	$A^4a^2 B^5b$	$3\ A^4a^2 B^4b^2$	$A^4a^2 B^3b^3$	$A^3a^3 B^5b$	$3\ A^3a^3 B^4b^2$	$A^3a^3 B^3b^3$
Segregation of F_1	non-segr.	non-segr.	399: 1	399: 1	399: 1	199: 1
Segreg. between F_1	4		5			1

SCHEDULE 4. *Calculations of the expected ratios of the cross, when two independent loci of the mother plant are postulated to segregate in simplex and triplex.*

Parents	$Aa^5 B^3b^3$ (simplex-triplex) × $A^6 B^6$ (hexaplex)							
Female gametes	$Aa^2 B^3$	$9\ Aa^2 B^2b$	$9\ Aa^2 Bb^2$	$Aa^2 b^3$	$a^3 B^3$	$9\ a^3 B^2b$	$9\ a^3 Bb^2$	$a^3 b^3$
F_1 plants	$A^4a^2 B^6$	$9\ A^4a^2 B^5b$	$9\ A^4a^2 B^4b^2$	$A^4a^2 B^3b^3$	$A^3a^3 B^6$	$9\ A^3a^3 B^5b$	$9\ A^3a^3 B^4b^2$	$A^3a^3 B^3b^3$
Segregation of F_1	non-segr.	non-segr.	non-segr.	399: 1	399: 1	399: 1	399: 1	199: 1
Segreg. between F_1	19			20				1

TABLE 1. *Frequency of F_1 plants producing indicated number of seedlings. Plants segregating in green and white.*

Number of seedlings:	0—100	101—200	201—400	401—600	601—1000	>1000	Total
Freq. of non-segr. plants:	9	4	3	1	2	12	31
Freq. of segr. plants:	—	2	1	4	2	18	27
							58

TABLE 2. *Obtained ratios of green and white seedlings compared with the expected segregation ratios of 399 : 1 and 199 : 1.*

Total numbers of seedlings observed			Hypothetical ratio: 399: 1			Hypothetical ratio: 199: 1		
			Expected values		P-values	Expected values		P-values
Green	White	Green / White	Green	White		Green	White	
3316	1	(3316: 1)	3308.71	8.29	0.02—0.01			
4294	2	2147: 1	4285.26	10.74	0.02—0.01			
1568	1	(1568: 1)	1565.08	3.92	0.3—0.2			
1431	1	(1431: 1)	1428.42	3.58	0.3—0.2			
2756	3	919: 1	2752.10	6.90	0.2—0.1			
5492	8	687: 1	5486.25	13.75	0.2—0.1			
3941	6	657: 1	3937.13	9.87	0.3—0.2	3927.26	19.74	0.01—0.001
2472	4	618: 1	2469.81	6.19	0.5—0.3	2463.62	12.38	0.05—0.02
2272	4	568: 1	2270.31	5.69	0.7—0.5	2264.62	11.38	0.05—0.02
562	1	(562: 1)	561.59	1.41	> 0.95	560.19	2.81	0.5—0.3
491	1	(491: 1)	490.77	1.23	> 0.95	489.54	2.46	0.7—0.5
465	1	(465: 1)	464.83	1.17	> 0.95	463.67	2.33	0.7—0.5
3616	8	452: 1	3614.94	9.06	0.9—0.8	3605.88	18.12	0.05—0.02
1350	3	450: 1	1349.62	3.38	> 0.95	1346.24	6.76	0.3—0.2
407	1	(407: 1)	406.98	1.02	> 0.95	405.96	2.04	0.8—0.7
1904	5	381: 1	1904.23	4.77	> 0.05	1899.46	9.54	0.2—0.1
348	1	(348: 1)	348.13	0.87	> 0.95	347.25	1.75	0.9—0.8
3251	10	325: 1	3252.85	8.15	0.7—0.5	3244.69	16.31	0.2—0.1
620	2	310: 1	620.45	1.55	> 0.95	618.89	3.11	0.8—0.7
1540	5	308: 1	1541.14	3.86	0.8—0.7	1537.27	7.73	0.5—0.3
1474	5	295: 1	1475.30	3.70	0.7—0.5	1471.60	7.40	0.5—0.3
785	3	262: 1	786.03	1.97	0.8—0.7	784.06	3.94	0.9—0.8
1181	5	236: 1	1183.03	2.97	0.5—0.3	1180.07	5.93	0.9—0.8
3115	18	173: 1	3125.17	7.83	< 0.001	3117.34	15.66	0.7—0.5
3265	23	142: 1	3279.78	8.22	< 0.001	3271.56	16.44	0.2—0.1
145	1	(145: 1)						
101	1	(101: 1)						

green, there is but a limited possibility for the father plant to have any segregating albino genes in common with the mother plant. Moreover, the chance for eventual non-hybrid plants to occur is very slight, as the selfed seedlings originating from a simplex plant must segregate in 3 green : 1 white. Thus, it is very likely that all the plants obtained from the cross have been true hybrids. Besides, all eventually obtained non-hybrid plants must, after isolation. give a duplex (24 : 1) or a simplex (3 : 1) segregation ratio, and, thus, be easily recognized in the study of the next generation.

Sixty F_1 plants have been isolated during the summers of 1955 and 1956. The majority of the plants have been surprisingly self-fertile and 58 plants have given seeds (Table 1), many of them abundantly. Twenty-seven of the plants have given white seedlings in their progeny and 31 only green ones. The segregation ratios of green and white seedlings of the selfed F_1 plants are given in Table 2. The majority of the segregating F_1 plants, *i. e.* all except two, have given ratios which fit

TABLE 3. *Frequency of segregating and non-segregating F_1 plants, which have produced an indicated minimum number of seedlings, compared with the segregation ratios of Schedules 2—4.*

	Segregating	Non-segregating	P-values
F_1 pl. found, >100 seedl./pl.	27	22	
Expected numbers (3 : 2)	29.40	19.60	0.5—0.3
» » (3 : 1)	36.75	12.25	0.01—0.001
» » (21 : 19)	25.73	23.27	0.8—0.7
F_1 pl. found, >200 seedl./pl.	25	18	
Expected numbers (3 : 2)	25.80	17.20	0.9—0.8
» » (3 : 1)	32.25	10.75	0.02—0.01
» » (21 : 19)	22.58	20.42	0.5—0.3
F_1 pl. found, >400 seedl./pl.	24	15	
Expected numbers (3 : 2)	23.40	15.60	0.9—0.8
» » (3 : 1)	29.25	9.75	0.06—0.05
» » (21 : 19)	20.48	18.52	0.5—0.3
F_1 pl. found, >600 seedl./pl.	20	14	
Expected numbers (3 : 2)	20.40	13.60	0.9—0.8
» » (3 : 1)	25.50	8.50	0.05—0.02
» » (21 : 19)	17.85	16.15	0.5—0.3
F_1 pl. found, >1000 seedl./pl.	18	12	
Expected numbers (3 : 2)	18.00	12.00	>0.95
» » (3 : 1)	22.50	7.50	0.1—0.05
» » (21 : 19)	15.75	14.25	0.5—0.3

significantly when compared with the ratios of 399 : 1 or 199 : 1. Many of the plants have not, however, given a sufficiently high number of seedlings to make it possible to obtain a statistically significant decision between the two mentioned ratios. There are still some plants fitting significantly only for one of the ratios in question. Thus, plants have been found, the segregation of which is explained only by the ratio of 199 : 1, *i. e.* the expected ratio of a triplex segregation in two independent loci. This fact indicates that the mother plant must contain albino recessives segregating in two loci, and must, consequently, be of a simplex-simplex, a simplex-duplex or a simplex-triplex constitution, following one of the Schedules 2—4.

The simplex-duplex constitution of Schedule 3 seems to be the most plausible one. This conclusion is drawn by comparing the obtained ratio of segregating and non-segregating F_1 plants with the expected values of the Schedules 2—4, as demonstrated in Table 3. In comparing the segregating and non-segregating F_1 plants it is, however, necessary that only such as have produced a sufficiently high number of seedlings are taken into consideration. The fact is that the non-segregating F_1 plants, which have given only a low number of green seedlings, might have been proved to be of a triplex constitution if a larger material had been available. On this account, the non-segregating F_1 plants will appear in excess in progenies in which the number of investigated seedlings has been too low. Thus, several calculations between segregating and non-segregating F_1 plants have been made, using a certain minimum number of investigated seedlings per plant to restrict the number of F_1 plants of each calculation.

In Table 3 it is demonstrated that the ratio of Schedule 2, giving 3 segregating: 1 non-segregating, does not seem to be a very plausible one, when compared with the obtained numbers of F_1 plants. Thus, the mother plant probably has not been of the simplex-simplex constitution. The two other ratios originating from Schedules 3 and 4 give both statistically significant probabilities. The ratio af 21 : 19, originating from the postulation of a simplex-triplex mother plant, seems, however, to be the less likely one. Here the non-segregating F_1 plants never appear in excess when compared with the expected numbers in spite of the fact that part of the non-segregating plants determined on a low number of seedlings must be of a triplex constitution. The third possibility, the one in Schedule 3, postulating a mother plant of the simplex-duplex constitution, gives a good fit between obtained and expected ratios. It gives an excess of non-segregating F_1 plants, at least when the F_1

plants determined on the minimum number of 100 seedlings are taken into consideration.

Two of the segregating F_1 plants have given very few white seedlings, proportionally too few to give a significant fit for the ratio of 399 : 1. The found ratios in question are 3316 : 1 and 4294 : 2. The existence of such a low proportion of white seedlings in the progeny of the F_1 plants might be explained in different ways.

The most plausible explanation is the postulation of a strong under-representation of white seedlings. Such a strong deficit of white seedlings has been found in certain progenies of known, earlier investigated material, when grown under extreme conditions. Especially in the harvest of last summer (1956) chlorophyll-deficient seedlings have been scarce compared with the corresponding materials harvested during earlier years. This fact is probably due to the very dry weather during the flowering season of the summer of 1956.

Another explanation of this very low proportion of recessives is, however, to assume the occurrence of chromatid assortment in a multivalent of a tetraplex F_1 plant (*cf.* MYERS 1944). Multivalents are somewhat more common in the synthesized *P. pratense* than in the strain of natural *P. pratense* under investigation. Thus, the above-mentioned explanation is a plausible one. A decision between these two given hypotheses cannot be made without further isolations of the plants during several years giving a more abundant material for the calculations.

Lastly, there is a third possibility that might be discussed. The father plant of the cross is a superhexaploid with $2n=44$. If one of these extra chromosomes were homologous with any of the segregating chromosome sextettes, ratios with a very low proportion of white seedlings would occur. This condition is a possibility that could be proved by determining the chromosome numbers of the segregating plants. This investigation has, however, not been done as yet.

OBSERVED SEGREGATION RATIOS OF GREEN AND YELLOWISH SEEDLINGS

The above described recessive genes for white seedlings are not the only ones for chlorophyll deficiencies segregating in the investigated material. Table 4 gives the obtained ratios between green and yellowish seedlings found among the F_1 plants. This material of Table 4 originates from the isolations of the F_1 plants during the summer of 1956 only.

TABLE 4. *Obtained segregation ratios of green and yellowish seedlings compared with the expected segregation ratios of 399 : 1, 199 : 1 or 381 : 19.*

Number of seedlings observed			Hypothetical ratio 399: 1			Hypothetical ratio 199: 1			Hypothetical ratio 381: 19		
Green	Yellow	Green Yellow	Expected values		P-values	Expected values		P-values	Expected values		P-values
			Green	Yellow		Green	Yellow		Green	Yellow	
4237	1	(4237: 1)	4227.40	10.60	0.01—0.001						
2825	1	(2825: 1)	2818.93	7.07	0.05—0.02	2811.87	14.13	$<$ 0.001			
1239	1	(1239: 1)	1236.90	3.10	0.5—0.3	1233.80	6.20	0.1—0.05			
950	1	(950: 1)	948.62	2.38	0.7—0.5	946.24	4.76	0.2—0.1			
3032	4	758: 1	3028.41	7.59	0.3—0.2	3020.82	15.18	0.01—0.001			
2043	3	681: 1	2040.88	5.12	0.5—0.3	2035.87	10.23	0.05—0.02			
1218	2	609: 1	1216.95	3.05	0.8—0.7	1213.90	6.10	0.2—0.1			
605	1	(605: 1)	604.48	1.52	$>$ 0.90	602.97	3.03	0.5—0.3			
1437	4	359: 1	1437.40	3.60	$>$ 0.90	1433.75	7.25	0.8—0.7			
3099	9	344: 1	3100.23	7.77	0.8—0.7	3092.46	15.54	0.5—0.3			
3430	14	245: 1	3435.39	8.61	0.1—0.05	3426.78	17.22	0.7—0.5			
1531	7	219: 1	1534.15	3.85	0.2—0.1	1530.31	7.69	$>$ 0.90			
407	2	204: 1	407.98	1.02	0.7—0.5	406.95	2.05	$>$ 0.90			
146	1	(146: 1)									
102	1	(102: 1)									
77	1	(77: 1)									
1875	28	67: 1							1812.61	90.39	$<$ 0.001
2549	75	34: 1							2499.36	124.64	$<$ 0.001
944	26	36: 1							923.93	46.07	0.01—0.001
430	16	27: 1							424.82	21.18	0.3—0.2
2423	91	27: 1							2394.59	119.41	0.01—0.001
145	8	18: 1							145.73	7.27	0.95—0.90
466	30	16: 1							472.44	23.56	0.5—0.3

Thus, it is not as abundant as the one used for the calculations of the green and white seedlings. From Table (4) it is obvious that the found ratios can be classified into two categories. The majority of the plants give a segregation ratio statistically fitting the simple and double triplex ratios of 399 : 1 and 199 : 1. Seven of the F_1 plants have, however, a much higher proportion of yellowish seedlings. These two categories of segregation ratios will be treated separately as they are assumed to originate from genes of different loci.

The F_1 plants of Table 4 giving the segregation ratio of 399 : 1 and 199 : 1 have usually not produced a sufficient number of seedlings to give a decisive significance for either of the ratios. However, one of the plants giving 3430 green : 14 yellowish seedlings most likely has a double triplex constitution. This possibility will seem still more plausible if the above-mentioned deficit of chlorophyll-deficient seedlings of the material of 1956 is taken into consideration.

The segregation ratios of the mother plants concerning the green and yellowish seedlings have never been stated. The ratios of the yellowish seedlings have usually not been determined in earlier investigated material as it generally has been impossible to obtain any interpretation of the inheritance.

From the obtained numbers of segregating and non-segregating F_1 plants of the material under discussion (Table 5) it is found that the non-segregating ones are in the majority. This stands in contrast to what is the case with the material when the segregation of green and white has been taken into consideration. On account of these facts, it seems most plausible that the mother plant has been of a duplex and not of a simplex constitution, regarding the yellowish genes. If, therefore, the mother plant is postulated to be of a duplex or duplex-duplex

TABLE 5. *Frequency of F_1 plants producing indicated numbers of seedlings. Segregating plants yielding green and yellowish seedlings.*

Number of seedlings:	0—100	101—200	201—400	401—600	601—1000	>1000	Total
Freq. of non-segr. plants:	8	4	3	2	3	15	35
Freq. of segr. pl. (rec. yellow):	1	1	1	1	1	11	16
Freq. of segr. pl. (interm. yellow):	—	1	2	—	1	3	7
							58

TABLE 6. *Frequency of segregating and non-segregating F_1 plants, which have produced an indicated minimum number of seedlings, compared with the segregation ratios of Schedules 5 and 6.*

	Segregating	Non-segregating	P-value
F_1 pl. found, >100 seedl./pl.	15	27	
Expected numbers (1 : 4)	8.4	33.6	0.02—0.01
» » (9 : 16)	15.12	26.88	>0.90
F_1 pl. found, >200 seedl./pl.	14	23	
Expected numbers (1 : 4)	7.4	29.6	0.01—0.001
» » (9 : 16)	13.32	23.68	0.9—0.8
F_1 pl. found. >400 seedl./pl.	13	20	
Expected numbers (1 : 4)	6.6	26.4	0.01—0.001
» » (9: 16)	11.88	21.12	0.7—0.5
F_1 pl. found, >600 seedl./pl.	12	18	
Expected numbers (1 : 4)	6.0	24.0	0.01—0.001
» » (9 : 16)	10.8	19.2	0.7—0.5
F_1 pl. found, >1000 seedl./pl.	11	15	
Expected numbers (1 : 4)	5.2	20.8	0.01—0.001
» » (9 : 16)	9.36	16.64	0.7—0.5

constitution the ratios of segregating and non-segregating F_1 plants would be 1 : 4 or 9 : 16, respectively (Schedules 5—6). Table 6 gives the comparison between obtained and expected ratios for the recessively segregating material. From that Table (6) it is found that the most likely segregation ratio is the one of 9 : 16, thus, indicating a mother plant of the duplex-duplex constitution, regarding the recessive yellowish genes.

Seven of the plants segregating in green and yellowish seedlings have given a very high proportion of chlorophyll-deficient seedlings. The ratio might be interpreted as a duplex segregation ratio of 24 : 1. Such a segregation would be found in non-hybrid plants. But in the study of green and white seedlings of the same material no non-hybrid plants, segregating as duplex (24 : 1) or simplex (3 : 1), have been found. Moreover, many of the yellowish seedlings occurring in the progeny from these seven plants have been very difficult to distinguish from the green ones and, when they have become somewhat older, many of them have been impossible to discern. The most plausible explanation of this grading colour scale among the seedlings, ranging from normal green over light green to yellowish, is to assume that a gene for chlorophyll deficiency manifests itself intermediately. The colour of the

SCHEDULE 5. *Calculations of the expected ratios of the cross, when one locus of the mother plant is postulated to segregate in duplex.*

Parents	A^2a^4 (duplex) × A^6 (hexaplex)		
Female gametes	A^2 a	3 A a^2	a^3
F_1 plants	A^5 a	3 A^4 a^2	A^3 a^3
Segregation of F_1	non-segr.	non-segr.	399: 1
Segreg. between F_1	4		1

SCHEDULE 6. *Calculations of the expected ratios of the cross, when two independent loci of the mother plant are postulated to segregate in duplex.*

Parents	$A^2a^4B^2b^4$ (duplex-duplex) × A^6B^6 (hexaplex)								
Female gametes	A^2a B^2b	3 A^2a Bb^2	3 Aa^2 B^2b	9 Aa^2 Bb^2	A^2a b^3	3 Aa^2 b^3	a^3 B^2b	3 a^3 Bb^2	a^3 b^3
F_1 plants	A^5a B^5b	3 A^5a B^4b^2	3 A^4a^2 B^5b	9 A^4a^2 B^4b^2	A^5a B^3b^3	3 A^4a^2 B^3b^3	A^3a^3 B^5b	3 A^3a^3 B^4b^2	A^3a^3 B^3b^3
Segregation of F_1	non-segr.	non-segr.	non-segr.	non-segr.	399: 1	399: 1	399: 1	399: 1	199: 1
Segreg. between F_1	16				8				1

seedlings will then depend on the proportion between normal and recessive alleles. Such an intermediate inheritance for a yellowish-green chlorophyll deficiency has been demonstrated in the autotetraploid *Dactylis glomerata* (BRIX and QUADT 1953). If it is postulated that the segregating F_1 plants are triplex and that the multiplex and simplex seedlings manifest themselves as yellowish to light green the expected segregation ratio would be 381 green : 19 yellowish or 20.05 : 1. This segregation ratio fits significantly for some of the plants but other plants have a strong deficiency for these yellowish seedlings. This fact may depend on the great difficulty of distinguishing between green and yellowish seedlings. The existence of intermediately manifested yellowish genes segregating in the same material as true recessive ones will provide an explanation of the fact that the segregation ratios between green and yellowish seedlings have very often been impossible to interpret.

When calculating the expected ratios the segregation has always been postulated to occur without chromatid assortment and without any linkage between the loci. Chromatid assortment during the segregation cannot be frequent, as the multivalent formation must be very unusual in the material. To reach any certain determinations about the occurrence of linkage between the studied loci is impossible owing to the small material of the present investigation. The postulated ratios of two independently segregating loci seem, however, to fit well with the obtained ratios of the two segregating pairs of recessive white or yellowish genes. Thus, there is not likely to occur any close linkage between these pairs of loci. In order to study whether any close linkage might occur between the white and the recessive or intermediate yellowish loci, Table 7 has been arranged. The calculated values for independent segregating loci of Table 7 have been obtained by using

TABLE 7. *Observed frequency of F_1 plants segregating for indicated genes compared with values calculated on the postulation of independent segregation. (Only the F_1 plants yielding more than 100 seedlings have been taken into consideration.)*

	White only	White and rec. yellow	White and int. yellow	Rec. yellow only	Int. yellow only	Non-segr.
Plant segr. in:						
Frequency of pl. found:	14	8	5	7	2	13
Calculated value:	16.13	9.07	4.20	6.05	2.80	10.75

the found proportions between segregating and non-segregating F_1 plants for the three different kinds of genes. As is demonstrated by Table 7 a close agreement has been obtained between found and expected values. Consequently, there does not seem to occur any close linkage between the three different types of genes. Thus, none of the five investigated segregating loci are closely linked, and at least some of them are most likely situated in different chromosome sextettes.

DISCUSSION

The study of the cross between the natural *P. pratense* of a simplex constitution and the synthesized one, originating from *P. nodosum*, has been made in order to obtain plants with a known genetic constitution, and to find out the mode of segregation occurring in the F_1 and the F_2 of the cross. During the present study it has been demonstrated that the segregation ratios of both F_1 and F_2 can be explained by postulating an auto-hexaploid mode of segregation. The ratios found in the progrenies of the F_1 plants are, as expected, of the triplex type, some of them triplex for one locus giving 399: 1, and the other ones triplex for two loci giving 199 : 1. The existence of double triplex F_1 plants indicates that the plant of natural *P. pratense* used for the cross must have been segregating in two loci for the investigated recessives. The plant is postulated to be of the simplex-duplex constitution regarding the albino recessives, and of the duplex-duplex, when dealing with the recessive yellowish genes. These postulations agree well with the found distributions of segregating and non-segregating F_1 plants.

The segregation ratio of the simplex mother plant was 103 green : 31 white. This segregation ratio does not give statistical significance for the expected ratio of an isolated simplex-duplex individual, which is 2.57 : 1. Nevertheless, many plants of *Phleum* segregating in green and chlorophyll-deficient seedlings have given a deficit for recessives. This phenomenon does not only appear in *Phleum*: it has also been observed in other grasses as, for instance, barley (NILSSON-EHLE 1922, HALLQUIST 1926). GUSTAFSSON (1940) points out the fact that the under-representation of albino seedlings in general is more common in material segregating also for xantha or viridis, than in that segregating for albinos only. The material of the present investigation has, as demonstrated above, several genes for different chlorophyll deficiencies. As a minimum two recessive genes have been found for albino, two

recessive ones for yellowish or viridis seedlings and one intermediate gene for yellowish. Moreover, only the genes represented in at least three recessive alleles in the mother plant can be detected by the method used. In such a family with so many genes for chlorophyll deficiencies it is very likely that some plants give a certain deficit of recessives, especially an inbred plant such as the mother used for the cross. Also certain triplex F_1 plants seem to have an under-representation of recessives (Table 2) although this cannot be statistically proved in the relatively small material (see above, p. 08).

Both the F_1 and the F_2 of the cross between natural and synthesized *P. pratense*, gives thus, ratios which are easily explained by postulating an auto-hexaploid mode of segregation. In the F_1 hybrids the genes of the *nodosum* chromosomes seem to segregate regularly with the ones of the *pratense* chromosomes. This segregation between the two kinds of chromosomes seems to occur without any discrimination between the homologous chromosomes of the sextettes. The auto-hexaploid mode of segregation is the same in the hybrid material as in the material of natural *P. pratense* earlier investigated (NORDENSKIÖLD 1953, 1954). Thus, there seems to be no detectable difference in the pairing affinity of the chromosomes originating from the hexaploid *P. pratense* or the diploid *P. nodosum*. This fact has been proved for several loci of the genomes, and it does not seem very likely that all the investigated loci would be situated in the same chromosome sextette.

Another interesting phenomenon of the material that must be mentioned is the fact that there actually occurs such a large number of genes for chlorophyll deficiencies in one and the same plant. Therefore, the Omnia plant, from which the investigated mother plant originated in the second generation of inbreeding probably contains still other genes for chlorophyll deficiencies. This fact stresses the observations of earlier investigations of the Omnia strain of timothy, in which it has been found that such genes for chlorophyll deficiencies must be very frequent (NORDENSKIÖLD 1953). It must be the intricate auto-hexaploid mode of segregation of the material which makes it possible for a well known commercial strain of timothy to hold such a high frequency of lethal genes. The mode of segregation makes it possible to maintain a constant heterozygosity of the individuals of the population, and the segregation of homozygous recessive lethals occurs only in a very low proportion. Consequently, the presence of these lethals does not reduce the vitality of the strain.

SUMMARY

(1) A cross between a natural hexaploid *P. pratense* of a simplex constitution for albino seedlings and a synthesized hexaploid *P. pratense*, originating from a diploid *P. nodosum*, has been performed. The cross has been made in order to obtain F_1 plants of a known genetic constitution and to study the mode of segregation of the hybrids.

(2) Both F_1 and F_2 of the cross have given segregation ratios easily explained by postulating an auto-hexaploid mode of segregation. Thus, the mode of segregation is the same in the hybrid material as in the one of earlier investigated natural *P. pratense*.

(3) Judging from the segregation ratios of the hybrid material compared with the ones of natural *P. pratense* there seems to be no detectable difference in the pairing affinity of the chromosomes originating either from the hexaploid *P. pratense* or from the diploid *P. nodosum*.

(4) The study of the hybrid material has revealed the fact that the mother plant of natural *P. pratense* used in the cross must contain at least five loci segregating for different types of chlorophyll deficiencies.

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THREE NEW GENES IN PISUM

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TWO NEW GENES OF THE N—Fa SEGMENT

IN 1941 a mutant plant with very broad stipules, leaflets and pods appeared in the variety Witham wonder. These characters were determined by the gene *lat* (=*latifolium*). The allele *Lat* of normal plants is completely dominant.

From the mutant plant the pure line no. 19, homozygous for *lat*, was raised. Fig. 1 (from left to right) shows shoots of Witham wonder (*Lat Lat*), line no. 19 (*lat lat*) and a line homozygous for *lat* and *st* (reduced stipules). Also, in combination with *st*, it is easy to score the *lat lat* genotype.

Lat was combined with other marker genes by several crosses. Independent segregation was found between *Lat* and the genes *Gp*, *I*, *M*, *Tl*, *Wa* and *Wb*. Crosses nos. 101/46 and 187/46 of Table 1, however, show that *Lat* belongs to the *N—Fa* linkage group. According to LAMPRECHT and MRKOS (1950), the gene *Le* is situated in the same chromosome as *N—Fa*. This is most probably true although *Lat* and *Le* give independent segregation (Table 1, crosses nos. 221/46 and 83/50).

The length breadth index of stipules and basical leaflets of *Lat*- and *lat*-lines have been measured. HÄRSTEDT (1950) found that these organs reached their definitive shape from the first node with a lateral axillary flowering shoot (here called node 1) and upwards (here represented by node 2).

In 25 plants of Witham wonder (*Lat Lat*) the mean indices of the stipules from nodes 1 and 2 were 1.89 ± 0.019, and from the basical leaflets 1.51 ± 0.042. The corresponding combined results from three different *lat lat*-lines, each represented by 25 plants, were 1.60 ± 0.011 and 1.26 ± 0.024. The analysis of variance given below shows that there is no significant difference between the stipule indices of consecutive nodes, whereas the leaflets of node 2 are slightly different from those of node 1. Actually they are somewhat more narrow.

In the variety Alnarps stens (a type of Non plus ultra) an aberrant waxless plant was found in 1941, from which line no. 51 was raised. In this mutant the pods, peduncles, stipules and the lower surfaces of the

Analysis of variance

Item	D.F.	Variance stipules	Variance leaflets
Total	199		
Between lines	3		
a. *Lat—lat*	1	3.32335	2.30879
b. Between *lat*	2	0.04846	0.34296
c. Within lines	196	0.01867	0.02180
d. Between nodes 1—2	4	0.02464	0.08751
e. Within nodes	192	0.01855	0.02043

Variance ratios.
Stipules; a : c=178.01***, b : c=2.60, d : e=1.33
Leaflets; a : d=26.38**, b : d=3.92, d : e=4.28*

leaflets were waxless. On the stems and sepals only a very slight cover of bloom was present. The upper sides of the leaflets were normally glaucous.

Crosses between line no. 51 and other »waxless» lines representing the genes *wa* and *wb* (WELLENSIEK, 1928 a and b), *wsp* (LAMPRECHT, 1939), and *wlo* (NILSSON, 1933) have produced entirely waxy F_1 hybrids

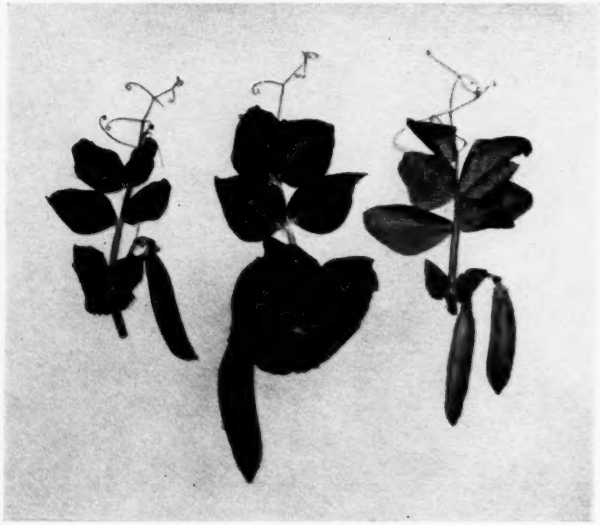

Fig. 1. From left to right, shoots of the genotypes *Lat Lat St St, lat lat St St, lat lat st st.*

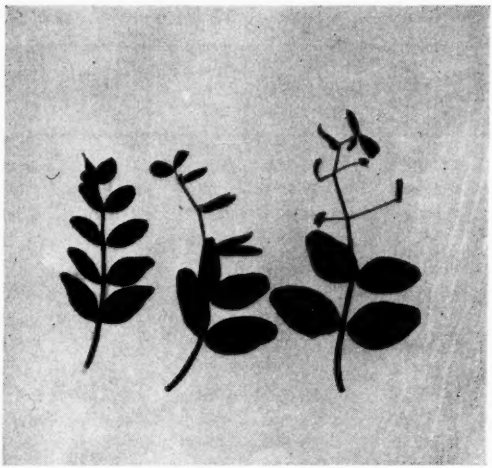

Fig. 2 From left to right, leaves of the genotypes $tl^W\ tl^W$, $tl^W\ tl^{pet}$, $tl^{pet}\ tl^{pet}$.

and F_2 progenies segregating, respectively, in the 9 : 7 ratios 132 : 92 ($\chi^2=0.65$), 94 : 83 ($\chi^2=0.71$), 38 : 36 ($\chi^2=1.14$), and, as regards *wlo*, in the 9 : 3 : 3 : 1 ratio 299 : 66 : 64 : 24 ($\chi^2=2.17$). The waxless gene of line no. 51 is thus a new independent member among the known genes determining the pattern of bloom. Phenotypically the expression is rather similar to that of *wa* (*cf.* LAMPRECHT, 1955 b) and the new gene has been called *was* (*s*=similis). This new gene gives independent segregation with *A*, *B*, *Fs*, *Gp*, *I*, *M*, *Oh* and *R*.

The linkage relations between *Was* and some genes of the *N—Fa* segment have also been studied. The crosses nos. 160/45 and 83/50 of Table 1 show that *Was* and *Fa* are closely associated. The inference of absolute linkage, given in the last column of Table 1 b, must be drawn with the greatest caution, since the detection of linkage has been made in the repulsion phase with only 430 individuals. Especially has cross no. 83/50 between line no. 90 (*le lat N fa Was*) and line no. 75 (*Le Lat n Fa was*) given valuable information which, however, is somewhat restricted due to a significantly disturbed *Fa* : *fa* segregation ($\chi^2=5.408^*$). The statistical analysis of this F_2 progeny has given the sequence:

$$Lat - 10.2 \pm 5.23\ \% - N - 19.4 \pm 2.38\ \% - Was$$

TABLE 1 a. *F_2 scoring of bifactorial crosses.*

Cross no.	Parental lines	Phase	Number of plants					Per cent germinated
			AB	*Ab*	*aB*	*ab*	*n*	
187/46	50×29	R	306	127	114	3	550	92
101/46	24×19	»	77	52	39	2	170	87
221/46	44×19	C	169	58	49	19	295	92
160/45	51×49	R	39	16	19	0	74	75
83/50	90×75	C	321	34	32	69	356	86
»	»	»	241	33	45	37	»	»
»	»	R	174	100	81	1	»	»
»	»	»	189	66	97	4	»	»
»	»	»	176	98	77	5	»	»
»	»	»	183	103	70	0	»	»
»	»	»	187	79	66	24	»	»
»	»	»	187	79	68	22	»	»
»	»	C	211	55	63	27	»	»
»	»	»	218	48	68	22	»	»
8/46	20×6	C	256	5	2	63	326	96
3/46	6×20	»	126	3	0	39	168	95
Total	»	»	382	8	2	102	494	

It ought to be mentioned that no significant linkage between any of these three genes and *Le* was detected in cross no. 83/50.

MULTIPLE ALLELES OF THE Tl LOCUS

Leaflets in place of tendrils, *i. e.* acacia leaf, is a recessive character caused by the gene *tl* (WHITE, 1917). In ordinary acacia plants the petiolule of the leaflets are quite short, but in 1945 a new acacia type with extended petiolule was found at Alnarp in the variety Witham wonder (see Fig. 2). If the original acacia allele is given the subscript *W* (=WHITE) and the new one *pet* (=petiolule), the conditions of dominance are of the sequence Tl—tl^W—tl^{pet}. *Tl* (tendril leaves as in Fig. 1) and tl^W are completely dominant to tl^{pet}, whereas the dominance of *Tl* over tl^W is only partial, since hybrids of the genotype $Tl\,tl^W$ have leaf like tendrils.

The pure line no. 20, homozygous for tl^{pet}, and raised from the new mutant, has been crossed with line no. 6 (*TlTl*) and no. 7 (tl^Wtl^W). In both cases monofactorial segregations were obtained, giving the ratio

TABLE 1 b. *Statistical analysis of bifactorial crosses.*

Cross no.	Factors *a*	*b*	χ^2 *a*	χ^2 *b*	χ^2 linkage	Per cent of crossing-over
187/46	*fa*	*lat*	3.782	0.442	30.73***	17.1 ± 4.11
101/46	*lat*	*n*	0.071	4.149*	18.02***	(18.6 ± 7.35)
221/46	*lat*	*le*	0.598	0.191	0.136	
160/45	*was*	*fa*	0.018	0.450	6.541*	0.0
83/50	*n*	*was*	2.157	2.936	129.44***	19.4 ± 2.38
»	*lat*	*fa*	0.734	5.408*	48.49***	(27.3 ± 2.85)
»	*lat*	*n*	0.734	2.157	40.45***	10.2 ± 5.23
»	*n*	*fa*	2.157	5.408*	22.01***	(22.7 ± 4.98)
»	*lat*	*was*	0.734	2.936	28.84***	22.6 ± 4.98
»	*fa*	*was*	5.408*	2.936	35.24***	(0.0)
»	*le*	*was*	0.015	2.936	3.271	
»	*le*	*n*	0.015	2.157	0.979	
»	*le*	*lat*	0.015	0.734	3.121	
»	*le*	*fa*	0.015	5.408*	1.743	
8/46	*r*	*tl*^{pet}	4.454*	2.982	284.5***	(2.3 ± 0.84)
3/46	*r*	*tl*^{pet}	0.286	0.000	144.9***	1.8 ± 1.04
Total	*r*	*tl*^{pet}	4.105*	1.968	437.4***	(2.1 ± 0.66)

Parantheses in the last column indicate dubious values due to disturbed single-factor segregation.

384 : 110 (χ^2=1.97) in the former and 312 : 68 (χ^2=10.23**) in the latter, with a significant deviation from 3 : 1 due to deficiency of plants homozygous for *tl*^{pet}.

LAMPRECHT (1955 c) gives the mean crossing-over value 5.4±0.16 % between *tl*^{W} and *r*. Table 1 (Total from crosses nos. 8/46 and 3/46) shows that the crossing-over value between *tl*^{pet} and *r* amounts to 2.1±0.66 %. This value is, however, somewhat uncertain due to a significant deviation of the *R* : *r* segregation of cross no. 8/46. In spite of this, the agreement between the two crossing-over values mentioned above further indicates that *tl*^{pet} belongs to the *Tl*-locus.

DISCUSSION

In the pea trial of the Horticultural Research Station at Alnarp the new mutants that incidentally appear are isolated in order to get new marker genes for the mapping of the *Pisum* chromosomes.

A remarkable feature of the *Pisum* chromosome map as known at the present time is the uneven distribution of the genes. In some chromosomes rather gene-crowded segments are separated by more or less empty regions. This is, among others, the case with the two chromosomes carrying the new genes dealt with in the present paper. Thus the *Lat—N—Z—Fo—Was—Fa* segment is rather crowded in comparison with other segments of this chromosome (*cf.* LAMPRECHT, 1955 a). In the *Gp—R* chromosome (LAMM, 1956), the segments Xa_1*—Pa—R—Tl* (LAMPRECHT, 1955 c) and *Cp—Gp—Cr—Cri—Te—Fs—U* (LAMPRECHT, 1953 a) are typically crowded. In one of the seven *Pisum* chromosomes no marker gene has so far been found (LAMM, unpubl.).

The expression of some genes is subject to internal and environmental influences and impaired vitality. Thus *fa* often gives disturbed segregations (*cf.* LAMPRECHT, 1956, p. 38). Here the new gene *was* is a suitable alternative in the mapping work. Besides, both *was* and *lat* may be easily recognized even in the juvenile stage which offers advantages to the investigator.

The genes *lat* and *fo* of the *Lat—Fa* segment both determine the length breadth indices of leaflets and stipules, but they have opposite effects. LAMPRECHT (1951) has shown that *fo* in combination with *red* gives extremely narrow leaves and seedless plants. The interactions of *lat* with *fo*, *red* and several other genes that might be of interest in this connection have not been investigated.

As mentioned on page 3 the intercrosses between some of the wax genes give 9 : 7 segregations. According to LAMPRECHT (1952 and 1953 b) this would suggest that duplications are involved. To some extent this may be true. EYSTER (1934, pp. 311—312) has pointed out that there are no fundamental differences between complementary (9 : 7) and duplicate (15 : 1) genes but that their dihybrid ratios alone do not prove that duplications of certain chromosome segments are involved. Only if similar sequences of such genes are found to be situated in non-homologous chromosomes or chromosome segments is there really good evidence of duplications.

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SUMMARY

In the present *Pisum* investigation the following main results have been obtained.

(1) In the *N—Fa* segment two genes, *i. e. Lat* and *Was* have been found. The order of these genes in relation to *N* is *Lat*— 10.2 ± 5.23 % — — *N*— 19.4 ± 2.38 % — *Was*.

(2) Plants homozygous for *lat* have very broad stipules, leaflets and pods and are easily recognized even in the juvenile stage.

(3) In plants homozygous for *was*, the pods, peduncles, stipules and the lower surfaces of the leaflets are waxless. Also this character is easily recognized in the juvenile stage. In linkage studies *was* is a suitable substitute for the gene *fa*.

(4) A new case of multiple allelomorphism has been found in *Pisum*, *viz.* $Tl—tl^{W}—tl^{pet}$. Homozygosity in tl^{pet} gives a new modification of the acacia leaf with extended petiolule.

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THE RECOVERY PHENOMENON AFTER IRRADIATION IN DROSOPHILA MELANOGASTER

I. RECOVERY OR DIFFERENTIAL SENSITIVITY TO X-RAYS

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INTRODUCTION

IN recent years it has been well established that the rate of chromosome aberrations induced by certain amounts and kinds of irradiation does not differ only between types of organisms but also between cells in the same organism, and between different physiological conditions. There are for example variations in sensitivity during the mitotic cycle as has been shown in Trillium by SPARROW (1951). In the post meiotic differentiation of *Drosophila* spermatozoa — spermiogenesis — it was shown (LÜNING, 1952 b) that different rates of aberrations were induced in cells irradiated at various stages and that this was due to differences in the number of primary breaks induced. The maximum rate was obtained from sperm irradiated in the spermatid stage. BAKER and VON HALLE (1953) showed in studies on dominant lethals that there was also a difference between the spermbatches of the first two days after irradiation. Eggs fertilized by sperm from the 1st batch hatches in a lower frequency than those fertilized by sperm from the 2nd batch. LÜNING (1954) confirmed their results and showed that there appeared fewer chromosome aberrations in the viable offspring from the 2nd than from the 1st spermbatch.

In order to explain this difference in the rates of dominant lethals BAKER and VON HALLE suggested that there was a preferential rejoining in the original order — recovery — of broken chromosome ends if the sperm was kept by the males for at least 24 hours. This suggestion was based on the fact that in one series where the males were withheld from mating the 1st day and were mated for the first time in

the period 24—48 hours after irradiation the rate of dominant lethals was the same as in the 2nd spermbatch. As was pointed out by LÜNING (1954) this conclusion is based on the hypothesis that the males keep their sperm when not mated. According to the results of LÜNING (1952 a) this is, however, not necessarily the case. Hence the hypothesis of a recovery of breaks cannot be considered as established on this material. In fact, as real differences in sensitivity were observed between other stages in spermiogenesis by analogy and in absence of proof of the contrary, it was supposed that also in this case there was a difference in sensitivity. That means, that different rates of breaks were induced.

By coincidence the discussion of this difference has been associated with the problem of the effect of the oxygen concentration during the irradiation. The reason for this combination is that BAKER and VON HALLE (1953) observed the difference between the 1st and the 2nd spermbatches after irradiation in air in an experiment designed to give information about the effect of the oxygen concentration on the rates of dominant lethals.

It ought to be emphasized that these two problems do not need to have anything in common although the two phenomena might be due to similar mechanisms. For that reason they ought to be discussed separately. To explain the dose-reduction effect of irradiation in anoxia, mainly two alternative explanations have been put forth which were reviewed by LÜNING (1954). They are identical with those presented above for the difference between the 1st and the 2nd spermbatch irradiated in air, viz. the differential sensitivity and the recovery hypotheses. Also in this case BAKER and VON HALLE (1953) supported the recovery hypothesis.

LÜNING (1954) pointed out that a final decision between the two hypotheses — differential sensitivity vs. recovery — could possibly be obtained from studies on ring-X chromosomes both when it concerns the oxygen effect and the difference between the 1st and the 2nd spermbatches irradiated in air. This statement is based on the idea that a proportion of the rejoined broken ends will join with the sister chromatid instead of with the »own» chromatid with a subsequent formation of a dicentric ring and elimination. If now the recovery hypothesis is correct a smaller difference between the first two spermbatches in the eliminations of X or Y from ring-X than from rod-X stocks would be supposed.

Thereafter an experiment along these lines appeared independently

from BAKER and VON HALLE (1955). Their results are in favour of the recovery hypothesis. They state that in one series there was no difference between the 1st and the 2nd batch while in the other there were slight differences. Between the air and nitrogen series they found slight differences in both series. The lack of differences between the 1st and the 2nd batch is remarkable as — although on this material not necessarily significant — differences would have been expected, as will be seen below. As the conclusion of a recovery process would mean something quite new in Drosophila genetics and as the consequences of such a fact might be of great importance for the further theoretical discussion of the mechanism of mutations it seemed worth while to repeat their experiments on a larger scale. It was soon evident that these studies were not sufficient and for that reason another technique was also used. The results of these experiments will be dealt with in this paper which is the first in a series.

MATERIAL

In the first group of experiments we intended to use the same technique as BAKER and VON HALLE, viz. eliminations of ring-X-chromosomes. To do this we used X^{c2};sc^8Y males. To make sure that the males had this constitution they were taken from a *yf*:=;$sc^8Y \times X^{c2}$;sc^8Y stock, which allows the control of the presence of the y^+ locus in the Y chromosome as accurately as possible. The X^{c2} chromosome was kindly put to our disposal by Dr. HANNAH-ALAVA. The X^{c2};sc^8Y males were mated to *y w sn* females. The normal offspring would in both sexes show wild type body colour. Individuals which had lost X^{c2} or sc^8Y and consequently were X0, would show yellow colour. Yellow males could also occur from loss of a part of the sc^8Y chromosome including the y^+ locus or from mutations in this locus. One can, however, suppose that these latter types of yellow males are rather infrequent compared to those due to the loss of whole X or Y chromosomes. In order to be sure that accidental non virginity of the *y w sn* females would not simulate X0 males and hence interfere with the true results, the males in the *y w sn* stock had the sc^8Y chromosome and had consequently wild type body colour. In the second part of the experiment, to be described below, males were used from a *yf*:=;$sc^8Y \times$Canton *S*;sc^8Y stock.

The males were X-rayed when 3—4 days old with 1620 or 3240 r given in 3 and 6 minutes respectively. They were subsequently mated

to *y w sn* females for 24 hours and were then transferred to new virgin females for another 24 hours. After this second mating period they were discarded. The females were allowed to continue egglaying for 2 periods of 3 days each. The number of normal females and males and of X0 males were recorded.

THE EXPERIMENTS

In the introduction a short review was given of the use of ring-X chromosomes to decide between the two hypotheses of recovery vs. differential sensitivity as explanations of the so called oxygen effect and the differences between the first two spermbatches after x-radiation in air. Here we shall extend this description of the principles and present them as formulas. In order to do so we must put forth some definitions and suppositions.

According to the »recovery hypothesis», in the two lots of sperm there are primarily the same rates of chromosome breaks, which under certain conditions later on could take part in interchanges. In the first lot all of them will remain available for interchanges while in the second a certain proportion will recover, that means rejoin in the original order and hence be withdrawn from the possibility to take part in interchanges, leaving the rest for possible interchanges.

In ring chromosomes one has to distinguish between two types of restitutions. One is the rejoining of a broken chromatid end with its previous partner, and the other is rejoining with the end of the sister chromatid. The latter type induced the formation of a dicentric ring which will be eliminated in subsequent divisions. In rod chromosomes on the other hand, both combinations will be normal. Breaks remaining open until they can take part in interchanges — MULLER (1940) supposed that this occurred at fertilization — are supposed to have the same chance of reunion in the two lots of sperm under consideration.

We can now compute the theoretical rates of eliminations in ring and rod chromosomes respectively in the two lots of sperm, using the following symbols:

n = the rates of breaks.
r = the proportion of recovery in the 2nd lot.
s = the proportion of restitution of breaks in the ring causing its loss.
p = the proportion of reunion at the time of the occurrence of interchanges.
q = (1—p) = the proportion of breaks remaining open.

Elimination of ring chromosomes.

Lot 1. $pns + qn$

Lot 2. $rns + p(1 - r)ns + q(1 - r)n$

Ratio: $$\frac{\text{lot 2}}{\text{lot 1}} = \frac{rns + p(1-r)ns + q(1-r)n}{pns + qn} =$$

$$= \frac{n(rs + ps - prs + q - qr)}{n(ps + q)} =$$

$$= \frac{ps + q + rs - prs - qr}{ps + q} =$$

$$= 1 - r + \frac{rs}{ps + q} \qquad (1)$$

Eliminations of rod chromosomes.

Lot 1. qn

Lot 2. $q(1 - r)n$

Ratio: $$\frac{\text{Lot 2}}{\text{Lot 1}} = \frac{q(1-r)n}{qn} = 1 - r \qquad (2)$$

From these calculations we see that in ring chromosomes one would expect a higher ratio between the two lots than from rod chromosomes as $\frac{rs}{ps + q}$ by definition is positive.

On the other hypothesis, differential sensitivity, the rates of primary breaks are different and will be called n_1 and n_2. It is supposed that there is no recovery. The corresponding calculations will be as follows.

Elimination of ring chromosomes.

Lot 1. $pn_1s + qn_1$

Lot 2. $pn_2s + qn_2$

Ratio $$\frac{\text{Lot 2}}{\text{Lot 1}} = \frac{pn_2s + qn_2}{pn_1s + qn_1} = \frac{n_2(ps + q)}{n_1(ps + q)} = \frac{n_2}{n_1} \qquad (3)$$

Elimination of rod chromosomes.

Lot 1. qn_1

Lot 2. qn_2

Ratio $$\frac{\text{Lot 2}}{\text{Lot 1}} = \frac{qn_2}{qn_1} = \frac{n_2}{n_1} \qquad (4)$$

On this hypothesis it is, therefore, to be expected the same rate of eliminations in ring and in rod chromosomes.

As has already been mentioned the males were taken from two stocks; viz. X^{c2};sc^8Y and Canton *S*;sc^8Y. After irradiation the males were mated to *y w sn* females and the F_1 offspring was analyzed. The normal offspring is grey. Loss of either the X or the Y-chromosome would give X0 males, phenotypically yellow. In an experiment of this type there also appear mosaics which might be mainly yellow but contain small grey areas. These cases were recorded as non-yellow flies irrespective of the smallness of the y^+ area. The occurrence of yellow males was taken as criterion of the elimination processes. As the principle here is a comparison between eliminations of ring and rod chromosomes we must remember that in the »ring»-stock it is only the X-chromosome that is ring shaped while the Y is a rod. This is of importance only in the discussion of the recovery hypothesis where a difference in the ratio between ring and rod chromosomes is expected. The ratio (1) obtained above presupposes that both sex chromosomes are rings. As only the X chromosome is a ring, the expected value will be between those in (1) and (2) depending on the relation between the rates of chromosome breaks induced in X and Y chromosomes.

The results of the experiments are presented in Table 1. Section *a* in this table includes the data from the ring chromosomes; and section *b* the data from the corresponding experiment with rodshaped chromosomes. Ratios to be considered in the further discussion are presented in section *c* of this same table.

We will first concentrate on the difference between the first two spermbatches which appear in irradiation in air. Similarity between the ratios from ring and rod-series would indicate that the cause is a differential sensitivity. On the other hand a higher ratio in rings than in rods is in favour of the recovery hypothesis. As is seen from Table 1 sec. c the figures are in agreement with the latter expectation. There is also agreement with BAKER and VON HALLE's results. We are hence forced to conclude, in contradiction to the analogous conclusion by LÜNING 1954, that the difference between the first two sperm batches is a result of a recovery process.

If we then turn to the oxygen effect and compare the ratios between the 1st spermbatches in the air and nitrogen series respectively, we see that the ratios in the ring series are slightly higher than that in the rod series. This difference is, however, not so conspicuous as was the case in the comparison between the two spermbatches after irradiation in air. From these data the conclusion cannot be made whether there *is* a recovery process or not.

TABLE 1. ***Induction of X0 males by irradiation of sperm rod or ring X chromosomes.***

The dose is given below. A stands for irradiation in air; N_2 for that in nitrogen and the figure for the mating period.

a) *y w sn*×X^{c2};*sc*^{8}Y
b) *y w sn*×Canton S;*sc*^{8}Y
c) Ratios between figures from a and b.

Series	Total	y-males Abs. n:r	y-males %
	a		
Control	11779	65	0.55
1620 A 1	10936	219	2.00
A 2	10145	178	1.75
1620 N_2 1	12543	202	1.61
N_2 2	8334	130	1.56
3240 A 1	4814	141	2.93
A 2	6904	185	2.68
3240 N_2 1	8866	200	2.26
N_2 2	7549	161	2.13
	b		
Control	17288	4	0.023
3240 A 1	4850	42	0.87
A 2	4554	26	0.57
3240 N_2 1	12694	68	0.54
N_2 2	5959	26	0.44

c

Ratios corrected for the spontaneous rates

Ring: $\frac{1620\ A\ 2}{1620\ A\ 1} = 0.90$ $\quad$ $\frac{1620\ N_2 1}{1620\ A\ 1} = 0.72$

$\frac{3240\ A\ 2}{3240\ A\ 1} = 0.83$ $\quad$ $\frac{3240\ N_2 2}{3240\ A\ 2} = 0.73$

Rod: $\frac{3240\ A\ 2}{3240\ A\ 1} = 0.65$ $\quad$ $\frac{3240\ N_2 1}{3240\ A\ 1} = 0.61$

The results by BAKER and VON HALLE (1955) in the series where they used *sc*^{8}Y are partly in agreement with the results in this paper. By this method we can consequently neither prove nor disprove the existence of a recovery phenomenon. To solve the problem we are forced to develop another technique which will be more decisive.

The studies of the oxygen effect in *Drosophila* have up till now been confined to comparisons between results from flies treated in air and in nitrogen. By these methods we have not been able to decide between

the recovery and the differential sensitivity hypotheses. For that reason we thought that a combined treatment, in air and in nitrogen, of the same flies would give us some information. By dividing the dose in two fractions, separated by an interval long enough to allow a change of atmosphere, it would be possible to give half the dose in air and the other half in nitrogen. This will be compared with the results of giving both treatments in air. The possibility of giving two treatments in nitrogen was abandoned as the flies should have had to stay too long in nitrogen. The nitrogen series in Table 1 sec. b is included for comparison. In the combined air-nitrogen treatment there are two possibilities, viz. that the first is in air and the second in nitrogen and vice versa. Hence we have four combinations.

A. 1620 r in air+15 min. air+1620 r in air
B. 1620 r in air+15 min. N_2+1620 r in N_2
C. 15 min. N_2+1620 r in N_2+15 min. air+1620 r in air
D. 15 min. N_2+3240 r in N_2.

Males from the Canton *S;sc*^{8}Y stock were used as the greatest differences should be expected by using rod-shaped chromosomes as is seen from Table 1 sec. c.

On the hypothesis of a differential sensitivity one would expect that the rates of eliminations of rod-X and -Y-chromosomes should be in the order A > B=C > D, with B and C about midway, from A and D.

The results are presented in Table 2.

TABLE 2. *Induction of X0 males after fractionated irradiation to the Canton S;sc*^{8}*Y males.*

A: 1620 r air+15 min air+1620 r air
B: 1620 r air+15 min N_2+1620 r N_2
C: 15 min N_2+1620 r N_2+15 min air+1620 r air
D: 15 min N_2+3240 r N_2

The figure after the capital letter is for the mating period.

Series	Total	y-males	
		Abs. N:r	%
A 1	17633	174	0.99
A 2	22837	168	0.74
B 1	20185	173	0.86
B 2	20333	100	0.49
C 1	7917	90	1.14
C 2	9263	59	0.64
D 1	12694	68	0.54
D 2	5959	26	0.44

It is immediately seen that as regards the 1st spermbatches the results are about the same in series A, B and C. A heterogeneity test gave $P=0.08$; heterogeneity test of A vs B+C gave $0.70 > P > 0.50$. This is not in agreement with the a priori expectation, $A > B=C$, according to the hypothesis of differential sensitivity depending on the oxygen concentration. The fact that the rates of elimination were nearly equal in all three series indicates that there is no detectable effect of irradiation in nitrogen, whether it has been given before or after in combination with irradiation in air. Thus it seems as if the irradiation in nitrogen was no less effective in producing chromosome breaks than irradiation in air. A consequence of this would be that in series D there should be induced as many breaks as in the other three series. In spite of this there is in series D a lower rate of elimination in the 1st spermbatch than in series A, B and C. This means that fewer breaks in the D than in A, B and C series are left open and are subsequently eliminated. There is a recovery.

There is at present no data controversial to the latter alternative and we will for that reason accept it as being the most satisfying explanation of the oxygen effect in *Drosophila* sperm from the 1st day.

From comparative studies on various types of mutations induced in the 1st and the 2nd spermbatch irradiated in air or in nitrogen atmosphere, we know that there is a larger difference after treatment in air than in nitrogen (BAKER and VON HALLE, 1953, and LÜNING, 1954).

The effect of the low oxygen concentration is stronger in sperm of the 1st than in those of the 2nd batch. We will now examine the series with half the dose given in air and half in nitrogen atmosphere, as regards the 1st versus the 2nd batch. It was stated above that in the 1st spermbatch there was no detectable effect of the treatment in nitrogen. Thus we might expect that the sperm of the 2nd spermbatch either did not show any effect, or that it showed an oxygen effect. It means that in the latter case there would be fewer eliminations than in the former. If we express it in another way, the ratio between the 2nd and the 1st spermbatches should be the same in series A, B and C, if there was no oxygen effect in the 2nd spermbatch while, if there was an effect, the ratio should be lower in B and C than in A. The results are not conclusive even if the rates in B 2 and C 2 are lower than in A 2. To get decisive results the experiment ought to be repeated in a much larger scale which we do not intend to do at present.

DISCUSSION

In this paper we have been dealing with the reason for the difference in effectiveness of irradiation depending on one hand on the oxygen concentration and on the other of the mating period, the 1st or the 2nd day after treatment. As was said in the introduction these two phenomena are independent but have by coincidence for the most part been discussed together. The reason for this is that the same material can be used in studies of both problems. Furthermore the mechanism of the two phenomena might be identical or similar and also for that reason it might be advantageous to compare them. In order to avoid confusion and to maintain a distinction between the two phenomena we will discuss them, in part, separately. As the most clear cut results have been obtained with regard to the oxygen effect the discussion in main will be devoted to this problem.

It was concluded above that our results are in agreement with BAKER and VON HALLE's (1953, 1955) suggesting that the same rate of primary changes is induced and that after irradiation in anoxia a certain proportion is withdrawn from the possibility to take part in rearrangements — there is a recovery.

The establishment of a recovery process raises a lot of questions pertinent to the understanding of this phenomenon. The first concerns the nature of the primary changes. Are these actual breaks or mere potential breaks, which if recovered, never open? Information about this may be obtained from the experiments with ring-X-chromosomes. The theoretical discussion leading to the formulae presented above was based upon the assumption that the breaks were primarily actual. On the other hand if it had been a question of potential breaks one would have got the same results as on the hypothesis of differential sensitivity. It was concluded that our results after irradiation in anoxia were between the two hypotheses while BAKER and VON HALLE's (1955) were more consistent with the recovery hypothesis. Thus the data seems to favour the idea that the primary change is an actual break.

Taking this for granted we will go over to the next question. What is the reason for the occurrence of recovery after irradiation in anoxia? BAKER and VON HALLE (1953) stated »the breaks in N_2 are more likely to rejoin and rejoin more quickly, than those induced in air». This would mean that there should be some inherent difference between breaks induced in the presence and in the absence of oxygen. If this was the case one would expect that in the fractionated irradiation se-

ries, in which half of the dose was given in anoxia, there would be a recovery of some of these breaks. Thus the rates of elimination in these series (B and C) should be between those in the series with the whole dose in air (A) and in anoxia (D). This is not in agreement with the finding that there was no heterogeneity between series A, B and C. Hence the results do not support the hypothesis that there is a different nature of breaks induced in air and in anoxia. As an alternative explanation it may tentatively be suggested that there exist factors which favour recovery but which are inactivated by irradiation in air with a dose of 1620 r while they are not inactivated by 3240 r in anoxia. This problem will be studied further and will be reported in a forthcoming paper.

The material presented also allows us to consider the reason for the difference between the 1st and the 2nd spermbatch after irradiation in air. As was stated in the introduction this problem is isolated from that of the oxygen effect, but the reason may be identical or similar in both cases. In the present investigation we have got the results as a by-product from the studies of the oxygen effect. However, it is only the data from the ring-X-experiments which allow any conclusions. It was found that there were only slight differences between the two spermbatches in these experiments. Such a result could be due either to the fact that there is no basic difference between the 1st and the 2nd spermbatch in the X^{c2};sc^{8}Y stock, or to a recovery in the 2nd spermbatch if we presuppose a primary difference between the two spermbatches. In order to decide between these an experiment has been performed using II—III translocations induced in sperm from X^{c2};sc^{8}Y males. This will be fully reported in a subsequent paper. Here it suffices to say that there were significantly more translocations in the 1st than in the 2nd spermbatch. Hence we can conclude that the results are in favour of the recovery hypothesis to explain the difference between the 1st and the 2nd spermbatch after irradiation in air.

The establishment of a recovery process by the ring-X-technique presupposes, as was fully discussed for the oxygen effect above, that the primary change was an actual break which later on recovered. We may then ask: when does this process occur? To discuss that we must put it in relation to the rejoining of broken ends in new orders — the occurrence of chromosomal rearrangements.

MULLER (1940) showed that induced chromosome breaks which later rejoined in new orders, remained open until fertilization. His conclusion was based on irradiation of sperm stored in females. We cannot a

priori suppose that this is true also for sperm kept by the males for up to 2 days after irradiation, all the more as OSTER (1955) showed that breaks in spermatids in pupae rejoin in new order prior to the moment of fertilization. Therefore, for a general discussion one must examine the 1st and the 2nd spermbatch as with regards to the rejoining of breaks in new orders. Such tests are made and will be reported in a forthcoming paper (LÜNING and JONSSON).

It remains to examine the breaks which will recover to see whether they will do so at a time different from that of the other type of rejoining. An experiment to study this question has been started (LÜNING and SCHUWERT).

Taking it for granted that a recovery process exists there appears another problem which only will be mentioned in short. If actual breaks are recovered after irradiation in anoxia, sperm giving viable offspring will under these conditions contain more primary breakpoints than in the case of sperm irradiated in nitrogen. According to the idea of LEA and CATCHESIDE (1945) and HERSKOWITZ (1946) the mere breaking will lead to the origin of recessive lethal mutations. On this basis one would expect a higher rate of recessive lethals in sperm irradiated in anoxia than in air. The reverse is the case (BAKER and SGOURAKIS, 1950, LÜNING, 1956). This problem will be dealt with in another paper in the series (LÜNING and SÖDERSTRÖM).

Finally we will call the attention to one more problem about the recovery process irrespective of whether it occurs in the sperm of the 2nd batch or after irradiation in anoxia. It concerns the recoverability of breaks. It was stated above that as regards the oxygen effect we could not demonstrate any difference between breaks induced in air and in anoxia. Still there remains the possibility that we have to consider two types of breaks, recoverable and non-recoverable. The present material does not allow any conclusion about these possibilities. We will, however, put it under debate because the establishment of »spontaneous» recovery will also possibly be followed by induced recovery by some kind of additional treatment.

SUMMARY

To explain the effect of oxygen concentration as well as the influence of the mating period on the rate of x-ray induced chromosome aberrations there have been two hypotheses proposed, differential sensitivity vs. recovery. By comparing the results obtained from rod versus

ring chromosomes it might be possible to decide between the two hypotheses. Canton S;*sc*^{8}Y and X$^{c^2}$;*sc*^{8}Y males were irradiated when 3—4 days old with 1620 or 3240 r in air or in N_2 and were subsequently mated to *y w sn* females for 24 hours. They were then transferred to new females for another 24 hours. The females were allowed to continue egglaying for two periods of 3 days each. The occurrence of supposed X0 males (phenotypically yellow) was recorded. The results are not conclusive as regards the oxygen effect but indicate that the difference between the mating periods was due to recovery.

In order to study further the oxygen effect the dose was subdivided in two equal parts with an interval of 15 minutes. By this technique it was possible to give the whole dose in air, half in air and half in N_2 and all in N_2. The results indicated that when half of the dose was given in air it was of no importance whether the second half was in air or in N_2. Hence it was concluded that primarily there are induced as many chromosome breaks in air as in nitrogen atmosphere. The results in the series with all N_2 gave fewer eliminations and consequently there has been some recovery.

The consequencies of these conclusions are discussed.

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THE RECOVERY PHENOMENON AFTER IRRADIATION IN DROSOPHILA MELANOGASTER

II. RECOVERY OF RECESSIVE LETHALS

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INTRODUCTION

THE fact that apparent gene mutations are many times associated with chromosome rearrangements, has led to the idea that all mutations are gross or minute structural changes and hence preceded by breakings of the chromosomes. This idea is nearly as old as the use of x-rays to induce mutations. Every now and then it is debated and data is presented in favour of or against it. The most radical view on this problem was presented independently by LEA and CATCHESIDE (1945) and HERSKOWITZ (1946). They suggested that the process of breakage itself caused the lethal mutation. Lethals not associated with detectable rearrangements were supposed to be restituted break points. HERSKOWITZ (1951) has modified his idea suggesting that there also existed recessive lethals which originated from pointmutations independent of breakage or that they were due to position effects.

LÜNING (1952) demonstrated that there was a much higher rate of chromosome aberrations induced in spermatids than in spermatozoa while the rates of recessive lethals showed a less difference. From these results it was inferred that some recessive lethals originated seemingly independent of chromosome breaks, intragenic changes.

From another field data has also been accumulated on the origin of recessive lethals, viz. from studies of the oxygen effect. BAKER and SGOURAKIS (1950) found that the rate of recessive lethals was considerably reduced after irradiation in nitrogen compared to that in air. LÜNING (1956) confirmed their results and discussed the reason for the fact that recessives and chromosome breaks were affected to about the same degree by the oxygen concentration during irradiation. It was

suggested that both chromosome breaks and intragenic changes were affected to the same degree by anoxia. This statement presupposed that the effect of anoxia was to change the sensitivity.

Meanwhile BAKER and VON HALLE (1955) have continued their studies on the effect of anoxia on chromosome breaks. From their results of supposed losses of ring-X-chromosomes they conclude that it is not a question of different sensitivity, but that a certain proportion of breaks is withdrawn from taking part in rearrangements (or being left open) by a process of preferential rejoining in original order — recovery. LÜNING and HANNERZ (in press) repeated the experiment with the same technique but were neither able to prove nor to disprove their conclusion. By using a fractionated irradiation technique they studied eliminations of rod X- and Y-chromosomes after irradiation with 1) two fractions in air, 2) one in air and one in nitrogen, and 3) the double dose in nitrogen. These results indicated that the rate of primary breaks or potential breaks were the same with irradiation in air and in nitrogen. When parts of the dose were given in air a recovery was prohibited, but when the whole dose was given in nitrogen, a proportion of breaks recovered.

This has some important consequences for the discussion about the origin of apparent gene mutations, e. g. recessive lethals. With irradiation in nitrogen it is supposed that there are as many primary breaks induced as with irradiation in air. A fraction of the breaks in the »nitrogen» series recovers, that means that those breaks rejoin in the original order, leaving the rest available for rejoining in new orders or remaining open (alternatively sister chromatid union). Due to the recovered chromosome breaks the sperm in the »nitrogen» series will contain more »primary break-points» per viable sperm than in the air series. On the hypothesis of a relationship between the process of breakage and the occurrence of recessive lethals more lethals are expected in the nitrogen than in the air series. This is contrary to the findings by BAKER and SGOURAKIS (1950) and LÜNING (1956). We are hence forced to conclude that there are no indications that breaks which undergo recovery will be associated with the origin of recessive lethals. This means that the breakage *per se* cannot give rise to lethal mutations and not either the process of reunion when it is the question of recovery.

These considerations are based on the results of BAKER and VON HALLE (1955) and LÜNING and HANNERZ (in press) both of whom used X0 males as indicators of chromosome losses. The fractionated irradia-

tion technique allows the use of any type of mutations in investigations in testing the recovery hypothesis. As the consequences of a recovery process on recessive lethals is of great importance we decided to repeat the test by using that type of mutation.

MATERIAL

In these experiments the MULLER-5 technique has been used. Males from a Canton S stock were irradiated when they were 3—4 days old. They were subsequently mated to MULLER-5 (sc^{S1} *B InS* w^a sc^8=M5) females for 24 hours and were then transferred to new virgin M5-females for another 24 hours. The impregnated females were allowed to continue egglaying for two 3-day periods. The F_1 females were tested in the conventional way. Cultures in which there appeared 12 or more M5 but not wild type males, were classified as lethals. If there were fewer than 12 M5 males another generation was produced.

The irradiation was done by a Skandia Intensiv apparatus operated at 170 kV, 15 mA and with an inherent filtration equivalent to 2 mm Al. The dose rate was 540 r/min. All cultures were kept in incubator at 25° ± 1° C.

THE EXPERIMENTS

The purpose of the present investigation was to confirm the findings of LÜNING and HANNERZ (in press) about the mechanism underlying the oxygen effect; this was done by using the fractionated irradiation technique, but on sex-linked recessive lethals. Thus this experiment was intended to be a repetition of that described in Table 2 in LÜNING and HANNERZ (in press). The results of these experiments are presented in Table 1.

It can immediately be seen that as regards to the 1st spermbatches the results in A, B and C are of the same order. A heterogeneity test gave P=0.30. This is in agreement with the findings of LÜNING and HANNERZ on X0 males, viz. that when half of the dose was given in air it was insignificant whether the second half of the dose was given in air or in nitrogen. The rates in the 2nd spermbatches, A 2, B 2, and C 2 show heterogeneity as in the test with X0 males, P=0.005. It is, however, remarkable that the lowest rate is in A 2, the series with both treatments in air. In spite of the heterogeneity we conclude that there does not seem to be any effect of irradiation in nitrogen when parts of the dose has been given in air.

TABLE 1. *The frequency of recessive lethals tested by the* MULLER-*5-technique.*

3—4 days old Canton S males were irradiated under four different conditions:

A: 1620 r in air+15 min. air+1620 r in air
B: 1620 r in air+15 min. N_2+1620 r in N_2
C: 15 min. N_2+1620 r in N_2+15 min. air+1620 r in air
D: 15 min. N_2+3240 r in N_2

The males were immediately mated to MULLER-5 females for 24 hours and were transferred to a new group for another 24 hour mating period. The mating periods are marked by the subscript 1 and 2 respectively to the letter of the series.

	Tested chromosomes	Recessive lethals	
		Abs n:r	Percent
A 1	1315	122	9.28
A 2	1283	75	5.85
B 1	1258	107	8.51
B 2	1280	115	8.98
C 1	1298	133	10.2
C 2	1268	89	7.02
D 1	1324	76	5.74
D 2	1306	69	5.28

Hence we can be allowed to pool the results in the three series, A. B and C. In doing so we get a mean for the 1st spermbatch of 9.35 % and for the 2nd of 7.28 %. These figures are in agreement with those presented by LÜNING, 1956, which were 8.92 % and 6.93 % respectively. If we then look at series D it is evident that irradiation in nitrogen atmosphere only, gives a lower rate of recessive lethals. These figures too are in agreement with those from LÜNING, 1956 (5.06 and 5.41).

By reasoning as LÜNING and HANNERZ the conclusion can be made from the preceding sections that irradiation in nitrogen does not produce fewer potential recessive lethals than irradiation in air, but that there is some kind of recovery when the whole dose is given in nitrogen. The consequences of this statement will be considered below.

DISCUSSION

We can now go back to the considerations of the origin of recessive lethal mutations which have been given in the introduction. In the light of the results of BAKER and VON HALLE (1955) and of LÜNING and HANNERZ (in press) it was stated that recessive lethals did not

originate by the process of chromosome breakage *per se* or by reunion in case of recovery.

In both experiments X0 males were used as indicators of the effect of x-rays under various conditions. This type of aberration cannot be studied further by progeny tests and consequently there is an uncertainty, especially as males phenotypically identical to X0 males can as well be the result of other types of mutational changes. Thus these results must be confirmed by other types of mutations among which we chosed sex-linked recessive lethals. In the preceding section it was shown that there was a good agreement between the results on X0-males and sex-linked recessive lethals. Hence, the reliability of the results on X0-males has been strengthened and consequently the conclusion about the independence of recessive lethals on the breaking of chromosomes *per se* and on the reunion by recovery. It ought to be emphasized that this statement does not mean that recessive lethals cannot be associated with chromosome breaks. This is too well established to be doubted.

Up to now we have been confined to the discussions about the possible relation between recessive lethals and chromosome breaks. We will now focus the attention to another aspect of the problem of the origin of recessive lethals. It concerns the mutation *process*. To make the concepts clearer we will use the terms the *original state* and the *mutant state* as designations of the starting point and the end point. Among the questions, which arise, is the question of whether the transition from the original to the mutant state occurs very quickly or whether it will consist of a stepwise change separated by longer or shorter intervals and how long these then will be?

The results presented above can give us some information about this problem. From the results in Ser. C 1 compared to Ser. A 1 it is clear that the same rates of recessive lethals are induced irrespective of the fact that the first part of the treatment in C was in nitrogen atmosphere. From this we can conclude that the rate of the primary changes induced by x-rays are independent of the oxygen concentration. As a corollary of this statement we must postulate that the same rate of primary changes as in Ser. A 1, B 1 and C 1 was also induced in Ser. D 1. The rate of recessive lethals was, however, significantly lower in the latter than in the former group. For that reason we are forced to conclude that some primary changes in Ser. D 1 have not resulted in recessive lethals. We therefore suggest that there has been some kind of recovery, that means a restoration of the *original state*.

It is possible that another non-lethal change is induced which may affect slightly the viability — detrimentals.

After the statement that the primary changes can be recoverable under certain conditions further questions about these conditions arise.

From the results in Ser. B 1 and C 1 it is clear that when half of the dose is given in air there will not be any recovery. Hence this process seems to be blocked by irradiation in the presence of oxygen irrespective of whether it precedes or succeeds the treatment in anoxia. To elucidate the dose dependence of this block-effect experiments have been started which will be reported in a forthcoming paper.

One other consequence of the statement of a recovery process is the question within which time a recovery will occur. The present data gives some information in regard to this problem. In Ser. D 1 we postulated the existence of a recovery process. If, after irradiation in nitrogen, this process was finished within 15 minutes, there would have been a recovery process also in Ser. C 1, giving recessive lethals at a rate between those in Ser. A 1 and D 1. But this is not the case, C 1 $>$ A 1 though not significantly. We are, for that reason, forced to suppose that no recovery happened within 15 minutes. How long the interval really is will also be studied in forthcoming experiments.

In the preceding sections we have drawn some conclusions pertinent to the question raised above about the mechanism of the mutation process. These can be summarized as follows: The primary change can be recoverable and stays so for at least 15 min.

It ought to be observed that this statement does not say what it is that has primarily changed, and to what degree it has changed. To start with the last problem we have to consider two alternatives.

I. The primary change immediately results in the mutant lethal state. Under certain conditions this change can be reversed giving a non-lethal state. Such a reversion would in fact be the same as a back mutation. If this idea was correct one would have to assume that a high proportion of new mutations mutate back immediately after the induction. In successive generations they are mostly known to mutate back with a very low frequency. That the possibility for a back mutation should be changed so enormously without changes in the state of the gene seems to be highly improbable. To explain such a case one would rather presuppose an unstable state but this is against the premises of this paragraph but will be identical with the following.

II. The primary changes give a potential lethal mutation — an unstable state — which at a later occasion either transforms to a

lethal mutation — a stable state — or to another non-lethal stable state (recovery). We cannot presuppose that recovery means restoration of the original state. It might be other new states which are non lethal but non the less more or less strongly deleterious to their carriers. Experiments to investigate this very important problem are under way and will be reported in another paper.

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SUMMARY

The rate of sex-linked recessive lethals induced by x-rays at different conditions was studied with the MULLER-5 technique. 3—4 days old Canton S males were given 3240 r. The dose was divided in two equal parts given with an interval of 15 minutes either at anoxia or in air atmosphere.

Ser A: Both treatments in air.
Ser B: The first treatment in air, the second at anoxia.
Ser C: The first treatment at anoxia, the second in air.
Ser D: The double dose (3240 r) at anoxia.

The males were immediately mated for 24 hours and were then mated to new females for another 24 hours.

The results showed that the rate of recessive lethals was about the same in the 1st spermbatch of Ser. A, B and C, while Ser. D showed a lower rate. It is concluded from these data that irradiation at anoxia and in air produce the same rate of primary changes of which a proportion will not result in recessive lethals if the whole dose is given at anoxia. Ser. D vs A; B; C. Hence the primary change can be recoverable and is so for at least 15 minutes. The consequences of this conclusion are discussed.

Furthermore the relation between recessive lethals and chromosome breaks is discussed and it is inferred that breakage *per se* and rejoining by a recovery process are independent of the occurrence of recessive lethals.

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THE RECOVERY PHENOMENON AFTER IRRADIATION IN DROSOPHILA MELANOGASTER

III. THE INACTIVATION DOSE OF THE RECOVERY PROCESS

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THE effect of irradiation of *Drosophila* sperm at the presence or absence of oxygen was discussed by LÜNING and HANNERZ (in press). They concluded from studies with fractionated irradiation in air or at anoxia that the same rate of primary chromosome breaks are induced but that when the whole of the treatment is at anoxia there is a certain rate of recovery — rejoining in the original order. This conclusion was, among other things, based on the fact that when half of the treatment was given at anoxia and the other half in air the result was the same as when both fractions were given in air. Each fraction was in that experiment 1620 r. This dose given in air was sufficient to block the recovery process. In order to study the influence of irradiation in air on the recovery process several experiments were made, which will be presented below.

MATERIAL AND METHODS

The same technique and the same stocks were used as in the experiment by LÜNING and HANNERZ, viz. *y w sn* females from *y w sn*$\times$ $\times$*y w sn*;*sc*^{s}Y stock and Canton S;*sc*^{s}Y (CS;*sc*^{s}Y) males from a *yf*:=; *sc*^{s}Y$\times$*CS*;*sc*^{s}Y stock.

The males were taken out when they were less than 24 hours old and were stored without females for 3 days. When they were 3—4 days old they were irradiated at anoxia (in N_2) or in air with one single dose or in two fractions 15 minutes apart. The males were immediately mated to *y w sn* females for 24 hours and were then in some series

mated to new females for another 24 hours. The females were allowed to oviposite for two 3-day periods.

The F_1 offspring was analyzed. The normal females and males had wild type body colour while loss of paternal X- or Y-chromosomes gave rise to yellow males. These losses were taken as indications of the occurrence of chromosome breaks.

The irradiation was done by a Skandia Intensiv apparatus operated at 15 mA, 170 kV, with an inherent filtration corresponding to 3 mm Al.

THE EXPERIMENTS

In the experiments by LÜNING and HANNERZ it was concluded that when half of a total dose of 3240 r was given in air, it was insignificant whether the second half was given in air or at anoxia. In order to confirm this finding we thought it worth while to repeat the experiment but to give a higher total dose, keeping the dose in air constant at 1620 r as in the previous test. The total dose was chosen to be 6480 r. For comparison there was also made one series with the whole of the dose given in air and another at anoxia. The results are presented in Table 1. It is evident that 1620 r given in air is sufficient to abolish the effect of anoxia. Hence it confirms the results by LÜNING and HANNERZ.

In order to get further information about the blocking of the recovery process a group of experiments was started. In these the total dose was kept constant but the proportion given in air was varied. The total dose was 3240 r and the dose rate was 540 r/min. Hence the duration of the treatment was 6 min. The irradiations in air varied from 5 sec. to full 6 min. There was also a series in which the whole of the dose was given at anoxia. This series was repeated once. The results are presented in Table 2 and Fig. 1. The dependence of the rate of

TABLE 1. *Offspring from crosses y w sn×CS;sc^{s}Y.*

3—4 day old males were given 6480 r. They were mated for 24 hours.

Ser. 1: 6480 r in air
Ser. 2: 1620 r in air+15 min interval+4860 r at anoxia
Ser. 3: 6480 r at anoxia.

		Yellow males	
Series	Total F_1 flies	Abs nr	%
1.	5709	147	2.57
2.	11292	304	2.69
3.	12378	228	1.84

TABLE 2. *Offspring from crosses y w sn×CS;sc^{s}Y.*

3—4 day old males were given a total dose of 3240 r at a dose rate of 540 r/min. In all but two experiments the dose was divided into two fractions of which one was given in air and the other at anoxia. The interval was 15 min. The proportion of the dose given in air was varied from 5 sec. to 5 min. In one series the whole of the dose was given in air and in another at anoxia.

Irradiation in			Yellow males	
Air	Nitrogen	Total F_1 flies	Abs nr	%
—	6′	12046	77	0.64
—	6′	21928	144	0.66
		33974	221	0.65
5″	5′ 55″	18912	117	0.62
15″	5′ 45″	18975	114	0.60
30″	5′ 30″	16553	104	0.63
1′	5′	12915	88	0.68
1′ 30″	4′ 30″	23747	180	0.76
2′	4′	13732	124	0.90
2′ 30″	3′ 30″	20640	164	0.79
3′	3′	17298	159	0.92
4′	2′	6504	64	0.98
5′	1′	6025	54	0.90
6′	—	14483	151	1.04

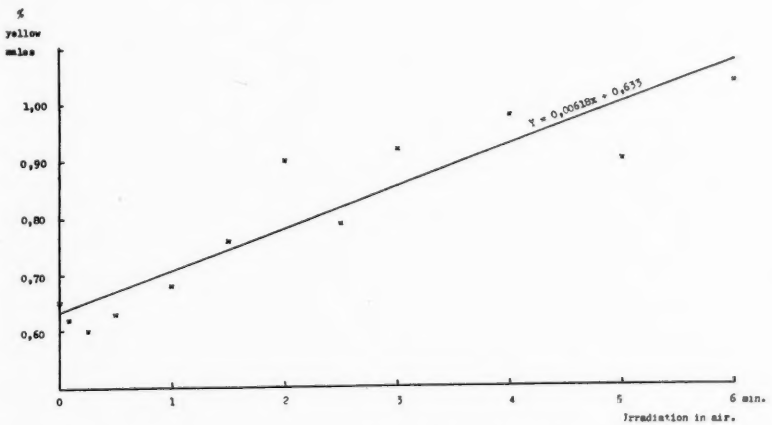

Fig. 1. The relation between the rates of yellow males and the time of irradiation in air (from Table 2). The units on the x-axis are=5 sec. Hence 1 min. is x=12, and so on.

TABLE 3. *Offspring from crosses y w sn×CS;sc^{s}Y.*

3—4 day old males were given 4320 r (8 min.). Mated for 24 hours.

Ser. 1: 8 min. in air
Ser. 2: 6 min. at anoxia (N_2)+15 min. interval+2 min. in air
Ser. 3: 8 min. at anoxia (N_2).

		Yellow males	
Series	Total F_1 flies	Abs nr	%
1.	10808	188	1.74
2.	7020	148	2.11
3.	11398	129	1.13

yellow males on the proportion of the dose given in air was studied. The regression line ($Y=0.00618x+0.633$) is drawn in the figure. There is not a significant deviation from this line. This is seemingly contradictory to the hypothesis that there is a blocking effect of anoxia by 1620 r (3 min.) in air.

Instead of repeating the series in the crucial region — about 2 to 3 min. irradiation in air — we decided to give a slightly higher total dose and thus get a wider difference between the limits — all the dose at anoxia *vs.* all in air. In the first experiment the total dose was 4320 r (8 min.). One group of males was irradiated in air, one at anoxia and one was irradiated at anoxia for 6 min. and after a 15 min. interval in air for another 2 min. The results are presented in Table 3. As is seen the 2 min. dose (1080 r) in air was sufficient to block the effect of anoxia. This experiment differed, however, from the previous ones with respect to the order of the two treatments. Here the treatment at anoxia preceded that in air. There are at present no indications that this would influence the result as is seen below.

TABLE 4. *Offspring from crosses y w sn×CS;sc^{s}Y.*

3—4 day old males were irradiated. They were mated for 24 hours.

Ser. 1: 540 r in air+15 min. interval+5940 r at anoxia (N_2)
Ser. 2: 5940 r at anoxia (N_2)+15 min. interval+540 r in air
Ser. 3: 5940 r at anoxia (N_2).

		Yellow males	
Series	Total F_1 flies	Abs nr	%
1.	5693	116	2.04
2.	5905	120	2.03
3.	7227	135	1.87

As a 2 min. dose (1080 r) in air was definitely effective when the total dose was 4320 r but was more doubtful in effectiveness when the total dose was 3240 r, it seemed possible that the inactivation dosages depended upon the total dose. For that reason one more experiment was made with a still higher dose, *viz.* 6480 r (12 min.). The effect of this dose in air and at anoxia is already known from the results in Table 1. In this experiment we gave 1 min. irradiation in air (540 r)

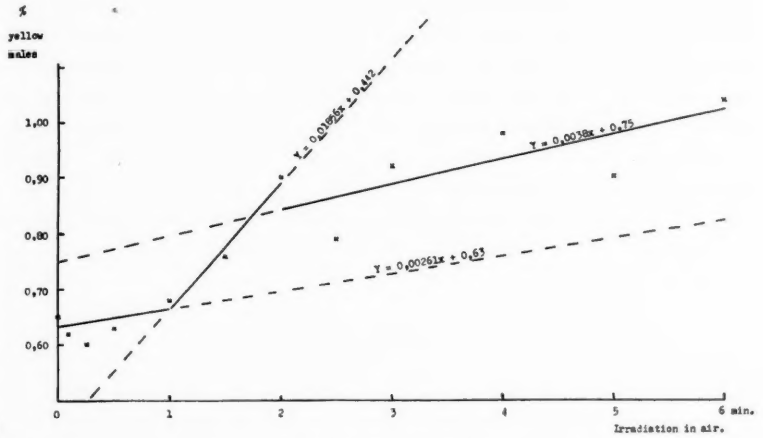

Fig. 2. Partitioning of the results from Table 2 in groups 0—1, 1—2 and 2—6. The reason for this is given in the text. The equations for the three regression lines are given in the figure. The units on the x-axis are 5 sec. Hence 1 min. is x=12, and so on.

and the remaining 11 min. at anoxia (5940 r). In one series the treatment in air preceded that at anoxia, in another it succeeded. In the latter series a sample of the males given 5940 r at anoxia were taken out prior to the second irradiation as a control of the effect of irradiation at anoxia only. The results are presented in Table 4. It is evident that a dose of 540 r (1 min.) was not effective in blocking the recovery process, there was no indication of such an effect even in a lower degree. Furthermore there is no evidence of an effect of the order of treatments whether that in air precedes ur succeedes that at anoxia.

Summarizing the results presented in Tables 3 and 4 it is shown that 1080 r (2 min.) can extinguish the effect of anoxia while 540 r (1 min.) is seemingly ineffective in spite of a higher total dose.

With this background we can return to the results presented in Table 2 and Fig. 1. There it was shown that the total material showed no significant deviation from a straight regression line. As the last two groups of experiments have shown that the crucial region is between 540 r and 1080 r one can subdivide the material in Table 2 in three groups: 0—1 min., 1—2 min. and 2—6 min. irradiation in air. For each group the regression lines have been calculated. They are presented in Fig. 2. It is evident that the regression line in the middle group, where the change was postulated to appear, was much steeper than the other two. Thus the true relationship between the rates of yellow males (chromosome eliminations) and the dose given in air seems to be a sigmoid function, with the point of inflection in the region between 540 and 1080 r. As the material does not differ significantly from a straight regression line the sigmoid function must be rather close to a straight line.

As concerns the biological meaning this proposed sigmoid function would indicate that the inactivation is a multi-hit process.

SUMMARY

In previous papers in this series it has been inferred that after irradiation of *Drosophila* sperm at anoxia there is a certain rate of recovery of chromosome breaks. It has also been shown that the recovery process could be blocked if parts of the treatment (1620 r) was given in air atmosphere. Here are presented further studies of the dose sufficient to inactivate the recovery-process.

3—4 days old Canton S;*sc*^{8}Y males were irradiated and were mated for 24 hours to *y w sn* females. The F_1 offspring was analyzed and the number of normal (grey) females and males as well as exceptional males (yellow=*y w sn*) were recorded. The exceptional males were supposed to be X0 having lost the paternal X or Y chromosome. There was firstly once more tested the effectiveness of 1620 r given in air. In this case the total dose was 6480 r. It is shown that 1620 r in air+4860 r at anoxia produced the same rate of aberration as when the whole of the dose was given in air. Furthermore it is shown that irradiation in air with 1080 r was sufficient, while 540 r was not sufficient to block the recovery process when the main part of the dose was given at anoxia. There is one experiment in which the total dose was 3240 r given at a rate of 540 r/min. The fraction given in air varied from 5 sec.

to full 6 min. The results do not show a significant difference from a straight regression line.

Based on the previous results that 540 r (1 min.) was not enough but 1080 r (2 min.) was sufficient to block the recovery process, the material in the last experiment has been subdivided into 3 groups, viz. 0—1 min., 1—2 min. and 2—6 min. irradiation in air, and the regression lines have been calculated for the 2 subgroups. It is evident that in the 1—2 min. interval the regression line is much steeper than in the other two intervals. This coincides with the a priori expectation. Hence one would conclude that the true function is a sigmoid one which, however, differs so slightly from a straight line that it cannot be detected by ordinary statistical methods.

The biological meaning of a sigmoid function is that the inactivation of the recovery process is a multi-hit process.

Acknowledgements. — The authors are deeply indebted to Miss B. SCHUWERT for technical assistance and to Mr. and Mrs. RAMEL for revising the English text.

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THE CHROMOSOMES OF THE MINK

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(Recevied May 22nd, 1957)

I. INTRODUCTION

THE first description of the chromosomes of the family *Mustelidae* was presented by KOLLER (1937), who reported a diploid chromosome number of 34 in the ferret (*Putorius furo*). SHACKELFORD and WIPF (1947) reported the diploid number of chromosomes in the mink (*Mustela vison*, PEALE and BEAUVOIS), to be 28. In the Japanese mink (*Mustela itatsi itatsi*) MAKINO (1948) found 38 chromosomes. The same chromosome number was observed by EHRLICH (1949) in the martes (*Martes foina*). MAKINO (1952) reported to have found 38 as the diploid chromosome number in *Helictis subaurantiaca*, SWINHOE (*Mustelidae*).

In a cytological study carried out recently on the testes of mink it has been found a chromosome number which is in disagreement with previous reports. The observed evidence will be presented here.

The animals examined were five farmbred minks of the wild type and of the mutant type Royal pastel. The material was collected during March 1956 and March 1957. The animals were stunned by a blow on the head and the neck was broken. The testes were removed immediately, cut into small pieces, and put into destilled water (15° C—25° C), where the pieces remained for about ten minutes, and were then put into either aceto-orcein or 50 per cent acetic acid, and fixed for about one hour. The pieces were then squashed and those which were fixed in 50 per cent acetic acid were stained after the Feulgen method (MATHEY, 1953). The aceto-orcein preparations were sealed with lactogelatin (ZIRKLE, 1947), and the Feulgen preparations were mounted in canadabalsam.

The photographs were taken on standard 35 mm Kodak Microfile film. Leitz 95× fluorite oil immersion objective, 15× and 30× periplan oculars were used. The magnifications are indicated on the figures.

II. RESULTS AND DISCUSSION

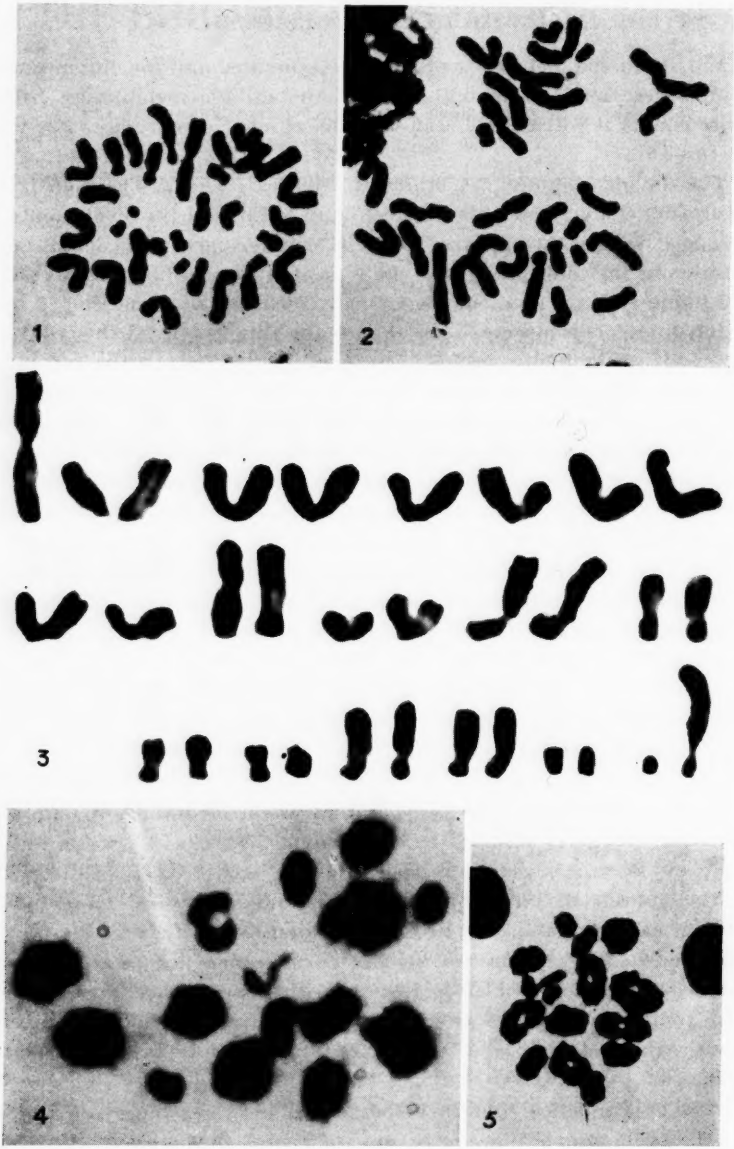
More than 200 metaphase plates were examined and the chromosome counts were made from both meiotic and mitotic metaphases. From these counts it was clearly seen that the number of chromosomes was 30 ($n=15$).

The diploid complex is pictured in Figures 1 and 2. The pairing of the homologous mates is shown in Figure 3. Each of the 30 elements is arranged with what appeares to be its counter-part, using length and position of the centromere as a guide. In that way 14 homologous pairs are found and one nonhomologous pair consisting of a rod-shaped and a dot-shaped chromosme. The latter is the smallest of all the chromosomes. It seems highly probable to speak of this pair as the sex chromosomes. The first 11 pairs consist of chromosomes with submetacentric or metacentric centromeres, two pairs consist of chromosomes with acrocentric centromeres, and in the remaining one pair the centromere cannot be located with certainty. The X-chromosome is acrocentric. The position of the centromere in the Y-chromosome cannot be located.

The first meiotic metaphase was found to exhibit 14 ordinary bivalents and the remaining element is thought to be the XY-complex (Figures 4, 5 and 6). The heteromorphic structure of the XY-complex is clearly seen in every meiotic metaphase, and it is noted that the Y-chromosome is paired with the tip of the X-chromosome. In some cases of meiotic metaphase it is found precocious movement of chromosomes (Figure 7) but there is no evidence of precocious condensation.

VENGE (1952) has described great variation in number of chromosomes of the germ cells of the male rabbit. The causes for the inconsistency in chromosome number may be ascribed to disturbances during mitosis or meiosis. The present author, however, observed in several cases variations in his material that evidently were due to the technique employed. In some cases chromosome groups were found which seemed to come from two cells pressed together. Groups were also found consisting of two or three chromosomes which apparently were separated from the rest of the chromosomes belonging to the same cell.

WALKER and BOOTHROYD (1954) concluded from their examination of the chromosome number in somatic tissue, that most of the deviation from the accepted diploid number were due to the squash tech-



Figs. 1—2, mitotic metaphases with 30 chromosomes. 1900×. — Fig. 3, pairs of homologous chromosomes. The XY complex below to the right. 2400×. — Figs. 4—5, metaphase of the first meiotic division with 14 ordinary bivalents and one XY-complex. (Fig. 4: 3600×. Fig. 5: 1800×.)

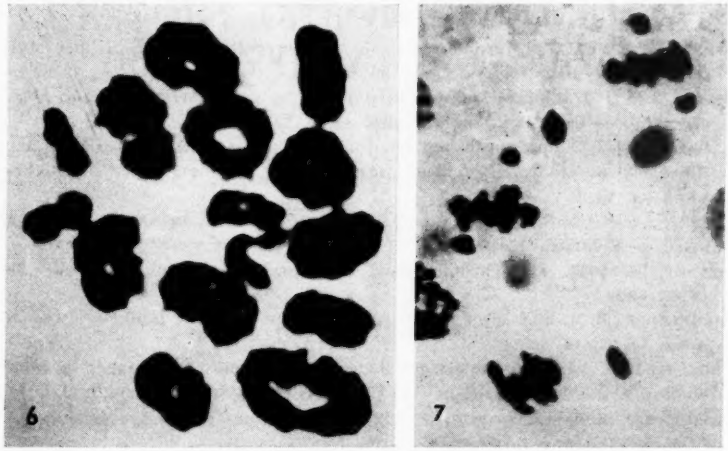


Fig. 6, first metaphase with 14 ordinary bivalents and one XY-complex. 3400×. — Fig. 7, meiotic metaphase. Two of the cells show chromosomes with precocious movement. 1000×.

nique. In the present study only those cells, which seemed to be intact with the chromosomes clearly visible and well spread, were counted. The results given were verified by chromosome counts from the endometrium of a pregnant female and from the spleen of a female foetus as well. The chromosome counts presented here are not in agreement with the description of the same species by SHACKELFORD and WIPF (1947). It seems evident that the karyotype of the mink observed in the present case is different from that of the Japanese mink (MAKINO, 1947), in spite of the close taxonomic relationship between the two species.

SUMMARY

More than 200 metaphases (meiotic and mitotic) from five different minks of the wild type and the mutant type Royal pastel were examined and the chromosomes counted. The diploid chromosome number was found to be 30 (n=15). SHACKELFORD and WIPF (1947) have reported 28 chromosomes in the same species.

The XY-complex was similar to the XY-complex described in other *Mustelidae* (KOLLER 1937, MAKINO 1948 and EHRLICH 1949).

Acknowledgments. — I wish to express my gratitude to Consul N. H. FLAATEN for his interest and to Dr. Ö. STRÖMNÆS and Mr. G. LIE for their kindly advices and criticism.

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ACCESSORY CHROMOSOMES IN THE ROOTS OF *POA ALPINA*

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INTRODUCTION

SINCE accessory chromosomes or B chromosomes were first reported to occur in some sexual diploid strains of *Poa alpina* (MÜNTZING, 1946) there has been undertaken a more detailed cytological study of their behaviour at meiosis and mitosis. In the recently published papers on that subject some characteristic features and peculiarities of these chromosomes are elucidated. MÜNTZING (1948) studied their properties and meiotic behaviour in p.m.c.'s, and found that although present in p.m.c.'s the accessories were almost completely eliminated from the root tips of the plant. All cells of root tips had 14 chromosomes regularly. However, in rare cases he observed accessories also in metaphase plates of the root tips. HÅKANSSON (1948), in his study of the embryology of *Poa alpina,* found accessories in the primary roots of germinated seeds derived from plants, which showed accessories at meiosis. But simultaneously a few cases of elimination of accessories in the main root were reported. He supposed that, as a rule, such elimination only occurred in the adventitious roots.

Chromosome behaviour at mitosis of *Poa alpina* has not been yet studied as much as at meiosis. As a supplement to the cytology of this species, a study of the chromosome numbers in primary and adventitious roots of germinated seeds and seedling progenies of two plants with B's at meiosis was undertaken. In addition, a number of clone plants derived from two old clones, known to possess accessories as a normal component of their meiotic chromosome sets, were used while studying accessories in adventitious roots.

Root tips of primary and of adventitious roots were fixed in chrome-acetic-formaline, sectioned and stained with crystal violet. Since the primary root tips are very slender and difficult to handle, at the sug-

gestion of Dr. ÖSTERGREN they were slightly stained with eosin in 95 % alcohol previous to embedding in paraffin.

OBSERVATIONS

In *Gramineae* the embryo possesses a few roots, *i. e.* this embryo possesses a primary radicule and one or two pairs of lateral rootlets which originate from the hypocotyl or from its vicinity. All these roots are commonly spoken of as primary or seminal roots. They form but a small proportion of the total root system of the grasses. Some or all of these roots die early in the development of the plant and are replaced by adventitious roots. The formation of numerous adventitious roots is associated with the phenomenon of tillering, *i. e.* the creation of a number of new shoots from axillary buds. Every tiller has procured its own independent system of adventitious roots arising from the first nodes of the shoot, within the soil or just above it.

Root tips from the germinated seeds of plant 37—6 were collected, successively as they appeared, and designated as the first, second and third primary roots. The length of the roots ranged from 5 to 15 mm.

A set of 33 metaphase plates sampled from the roots of 11 seedlings was examined. Frequency distributions of the number of chromosomes per plate is recorded in Table 1.

TABLE 1. *Number of chromosomes in the primary roots 5 to 15 mm long.*

Primary roots	Number of chromosomes								
	14	14+1	14+2	14+3	14+4	14+5	14+6	14+7	n
1st root		1	—	2	6	2	5	1	17
2nd root	1	2	1	4	—	2	2	1	13
3rd root	1			2					3
Total	2	3	1	8	6	4	7	2	33

One to four counts were made from each root depending on the clearness of the plates. All 33 counts, except two, (one in the third and one in the second primary root) showed the presence of accessory chromosomes. The total number of chromosomes ranged from 15—21, *i. e.* 1—7 accessories respectively. Fig. 1 is a plate from a second primary

root 12 mm long having 21 chromosomes (14+7). The plates in the same root often showed different chromosome numbers. Two plates, one in the second and one in the third root and derived from two different seeds, had 14 chromosomes only and were, thus, without accessories. They were found in root tips in which other plates had several accessory chromosomes. For instance, the plate with 14 chromosomes from the second root was seen in a root tip in which 3 other plates had 15, 15 and 16 chromosomes. The corresponding counts for sister plates of the plate without accessories in the 3rd primary root were 17 and 17 chromosomes. After comparison of the counts of the first and of the second primary root from the same germinated seed, the following figures were obtained:

Seedling No. 15 in the first root 18 and 19, in the second 17, 17, 19, 19
» » 20 » » » » 18 » 20, » » » 17, 17

Plates with 6 and 7 B's were found in the first and in the second roots as well (Table 1). In 19 plates, *i. e.* in more than 50 % of all plates, 4—6 accessories were present. The third primary root is represented by only 3 plates. This is due to the fact that in a number of cases the young seedlings from which the first and second primary roots were cut off did not grow longer, neither did they give the third root on the blotting paper. In addition to this, some germinated seeds deprived of their first or of two first root tips were transplanted to the pots in order to obtain later adventitious roots. A number of plates were not sufficiently distinct to enable counting of chromosomes, and were discarded. But in addition to the counts reported in Table 1, it was possible to see on 16 other plates that accessories were obviously present, since more than 14 chromosomes were to be seen. Of these plates six belonged to the 3rd root.

It was of interest to study, whether there was a decrease in the number of chromosomes correlated with the increase of the length of the primary root as a consequence of continued cell division, and caused by a successive elimination of accessories. For this investigation the seeds of another plant of *Poa alpina*, No. 12—7, were used. From germinated seeds the roots were allowed to grow to a length of 40—60 mm, and their root tips were then cut off and metaphase plates observed. To ensure the obtaining of 3—4 root tips of such a length, newly germinated seeds were placed with young roots in a nutrient solution, where they continued to grow until the desired length was obtained. They were cut successively as explained above and then analysed.

TABLE 2. *Number of chromosomes in the primary roots 40 to 60 mm long.*

Counts in primary root	Number of chromosomes							
	14	14+1	14+2	14+3	14+4	14+5	14+6	n
1st root				4	3	1	1	9
2nd root		1	2	3	—	2	—	8
3rd root			1	3	—	1	2	7
4th root			1	1				2
Total		1	4	11	3	4	3	26

From 7 plants 26 good plates in all were found. It was possible to make counts in 9 plates of the first, in 8 plates of the second and in 7 and 2 plates in the third and fourth primary roots respectively. From Table 2 it may be seen that the plates had from 15—20 chromosomes, including 1—6 accessories.

Plates with only 14 chromosomes have not been observed in this case. Accurate counts of other plates were not possible, but it was obvious that there were more than 14 chromosomes.

Chromosomes in adventitious roots were studied in a material derived from two old *Poa alpina* clones, designated as Sofia 022—1 and Sofia 022—2, as well as in four young clones raised from seedlings of plant 37—6. These latter ones originated from germinated seeds of which the first primary roots were used for the studying described above. The tips of adventitious roots were cut off from young lateral shoots of clone plants at the time of shooting, in late summer. The length of the adventitious roots was measured, and ranged from 1—150 mm. Roots of various length were fixed in order to see whether the length had any connection with the chromosome number. Young tillers at the time of tillering were transplanted each to a separate pot, or they were taken to nutrient solution where the roots continued growing until the desired length was obtained.

Counts were made on 45 plates. But in spite of a great variation of root length and the diverse origin of the clones used, it was not possible to find any difference in chromosome number in adventitious roots. As a rule, all the plates showed clearly only 14 chromosomes without any accessories. The young adventitious roots, just broken out from their

stem and scarcely perceptible, were found to have the same chromosome number as the roots, being 150 mm long, each having 14 chromosomes and no accessories.

Nine of the 45 chromosome plates belonged to roots the length of which did not exceed 1 mm. Fig. 2 represents such a plate from an adventitious root. The greatest class was that of 15—150 mm with a frequency of 19. The other 17 plates were placed in 3 intermediary classes.

As a result, it is evident that the elimination of accessories from adventitious roots had taken place at an earlier stage of root development, in any case, before the roots had emerged from the shoot. To see how

Figs. 1—4. Somatic plates (×2500). — Fig. 1, from a second primary root, $2n=14+7$. Fig. 2, from an adventitious root 1 mm long, $2n=14$. Fig. 3, from a root primordium, $2n=14$. Fig. 4, from a shoot primordium, $2n=14+4$.

the elimination occurs it would be necessary to study the mitotic divisions in the primordia of the adventitious roots, in the interior of the stem of a tiller. An attempt, using cross-sections through the shoot base, from which the adventitious roots break out, did not give good results. With longitudinal sections, however, numerous divisions in root primordia within young shoot were seen, but with a small number of metaphase plates sufficiently distinct to enable counts. The cell size in these sections were still smaller, and the chromosomes more crowded than was the case with the seminal root tip plates. Only in 21 of 52 young tillers sectioned countable plates were found. In some sections good metaphases were observed in root primordia, and in others in the primordia of the shoot.

Among 21 counts from 12 tillers only 2 plates, both of them from the same root primordium, were found to contain 16 chromosomes each. All other plates had but 14 chromosomes. One such plate is shown in Fig. 3.

In the shoot primordium there were found on the contrary 10 plates,

every one of which contained 15—18 chromosomes, *i. e.* 1—4 B's. Fig. 4 shows one of these having 18 chromosomes.

The fact that accessories were not detected in adventitious roots, whether long or very young and short, nor in primordia of adventitious roots in the interior of the stem, is in accordance with HÅKANSSON's (1948) assumption that elimination of accessories probably takes place at a very early stage of root development. The two plates from a root primordium, found in this investigation to possess 16 chromosomes, probably belong to that early stage, not yet having lost their accessories. At any rate, it seems that the solution of the problem will be found by investigations of the first cell divisions of the pericycle layer, which give rise to adventitious roots.

In the case of *Poa alpina* it was noticed that the adventitious roots were longer and thicker than the primary roots. Probably the same situation is met with in other grasses. If one takes root tips for cytological study from an older *Poa* plant which has reached its tillering stage, the majority of the tips will certainly belong to the adventitious roots, *i. e.* to the roots lacking accessory chromosomes. The reason for this is simply the prevalance of adventitious roots in the root system of adult grass plants. At the same time, owing to their greater size, they stand more chance of being collected and fixed than the primary roots.

Acknowledgement. — The work reported was carried out while the author was a Rockefeller fellow at the Institute of Genetics, Lund, Sweden. I am much indebted to Dr. MÜNTZING, Professor of Genetics at the University of Lund, for his helpful suggestions and interest during the period of this study.

SUMMARY

Chromosomes in the roots of *Poa alpina* plants with accessories in p. m.c. were studied with regard to the presence or absence, respectively, of accessories. In all primary roots, regardless of their length and development stage, accessories were present. On the other hand, these were completely eliminated from the adventitious roots, irrespective of their length. In addition, the lack of accessories was recorded also in young primordia of adventitious roots in the interior part of the stem.

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UNEQUAL CROSSING OVER IN THE BAR REGION OF DROSOPHILA MELANOGASTER

I. HIGH TEMPERATURE AS A MEANS OF INCREASING THE FREQUENCY

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THE same net amount of crossovers could result in three different ways (WHITTINGHILL, 1955): 1. random meiotic crossing over — the conventional theory of crossing over; 2. conditioned meiotic crossing over, caused by a weakening at a certain point of a chromosome in a gonial cell and resulting in a conditioned crossing over at the same point in all oöcytes originating from this cell — thus a gonial predisposition for meiotic exchanges in certain points; 3. fulfilled crossing over in a gonial cell with subsequent multiplication of the crossover chromosomes. The two last-mentioned possibilities would both result in a non-random distribution of crossovers, appearance of clusters, but in the conditioned meiotic crossing over the complementary classes would be equal, in gonial crossing over they might vary according to the uneven multiplication of the gonial cells. In experiments with X-rays and γ-irradiation of *Drosophila* females WHITTINGHILL (1955) obtained results which were in accordance with the hypothesis of gonial crossing over as the cause of increased crossover production by irradiated females. Using the data by FRIESEN (1936) he could also show that the induced crossing over in irradiated *Drosophila* males was problably of the same type.

It is not known whether the induction of crossing over by high temperature treatment is caused by the same phenomenon, although the appearance at equivalent times after treatment makes it probable (MAVOR and SVENSON 1924). It is also unknown whether gonial crossing over can occur in parts of the chromosomes outside the limited segments around the centromere in chromosomes II and III, where induced

TABLE 1. *Unequal crossovers in controls and different broods of females exposed to 31° C. for 48 hours.*

n = total number of offspring; UCO = number of unequal crossovers.

	Days after start of exposure	Number of vials	n	UCO	%
Control 1		30	14545	20	0.138
» 2		29	10935	13	0.119
Total		59	25480	33	0.1295
Exp. 1		37			
brood 1	3— 5		4304	4	0.093
» 2	6— 7		7330	14	0.191
» 3	8—10		7038	10	0.142
Exp. 2		31			
brood 1	6— 8		3789	8	0.211
» 2	9—11		4768	12	0.252
Exp. 3		30			
brood 1	6— 8		3004	9	0.300
» 2	8—10		5218	11	0.211
» 3	11—13		4868	6	0.123
Total		98	40319	74	
Brood 1: 1 and 3: 3 excluded		98	31147	64	0.2055

t-value for difference between Exp. and Control: 2.21, P 0.05—0.02

crossing over is clearly demonstrated. In the X-chromosome MAVOR (1923) found a pronounced crossing over decrease in the section *white—miniature* following X-ray treatment, but this could not be confirmed by MULLER (1926), who instead found a decrease in the most distal end of the X-chromosome, *scute—white*, and an increase in the proximal end, *Bar—Beadex*, but no change in the rest of the chromosome. STERN (1926) states that high temperature increases the crossing over frequency in the *Bar—bobbed* region, and KIKKAWA (1934) registers a small decrease in the *yellow—crossveinless* region after exposure to 31° C.

The occurrence of unequal crossing over in the Bar segment of the X-chromosome may be used in investigations concerning these problems. The result of such unequal crossing over in a homozygous Bar-female is either reverted + or *BB* (double-Bar) crossovers. If clusters of such types appear in the offspring they must be attributed to real gonial crossing over, as it is impossible that a gonial weakening of a chromosome could predispose to an unequal pairing in the following meiosis. STURTEVANT (1928) has shown that the spontaneous occur-

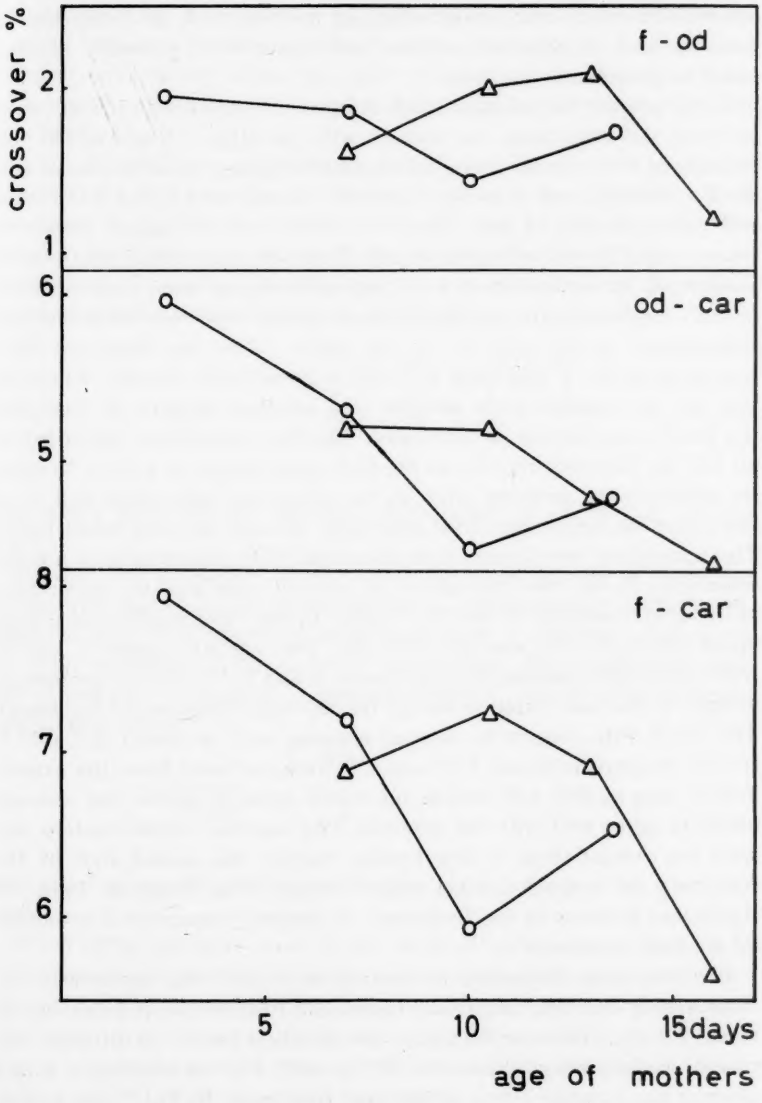

Fig. 1. Crossover frequency in different broods. Triangles: offspring from females exposed to 31° C for 48 hours, average age at start of exposure 4 days. Circles: offspring from control females.

rence of reverted+and of *BB* offspring ineracts with the frequency of crossing over in adjacent sections and therefore is probably not the result of gonial crossing over.

In the present investigation high temperature has been used as crossing over inducing agent on females with genotype *f B od car*/+*B*++ outcrossed to *f*+*od car* males. All normal offspring from this cross will be *B*/+ females and *B* males. Unequal crossing over in the Bar region will give offspring of four types: reverted+eyes combined with *f* or *od car* and *BB* eyes with *f* or *od car*. Three separate experiments were performed. In each of these 1—7 days old females were placed 5 by 5 in vials, provided with the usual corn-melasses food and subjected to a temperature of 31° ± 0.5° C for 48 hours. After the treatment they were kept at 25° C and each vial was supplied with 5 males. The eggs laid by the females were divided into separate broods by changing the food every second or third day. The first brood was discarded in all but the first experiment, as the high temperature is known to exert its influence on crossing over in the offspring from eggs laid 6—9 days after the beginning of the treatment (MAVOR and SVENSSON 1924). The subsequent two broods were classified with respect both to normal crossovers in the two regions *f—od* and *od—car* and the occurrence of unequal crossovers in the Bar region. In the third experiment a later brood (days 11—13) was also included. Two control crosses (1 and 2) were performed parallel to experiments 2 and 3. They were performed in exactly the same manner except for the high temperature treatment. The result with respect to unequal crossing over is shown in Table 1. In the comparison brood 1: 1 and 3: 3 were excluded from the experimental data as they fall outside the stated sensitive period and also are found to agree well with the controls. The angular transformation was used for computation of the t-value, taking the square root of the frequency as normal deviate with variance $^1/_{4n}$ (MATHER 1946). A significant increase in the frequency of unequal crossovers is produced by the heat treatment.

The crossover frequency in the region *f—car* was apparently the same among the offspring from control and heat treated females (6.8 % versus 7.1 %). However the age of the female is known to influence the crossover frequency (BRIDGES 1927), and this circumstance might obscure the possible effect of the heat treatment. In Fig. 1 the broods from different experiments have been combined according to age of the mothers, assuming 4 days average age at the start of the heat treatment. The control material has been correspondingly divided. In the

broods where the influence on unequal crossing over was established a definite crossover excess can also be seen both between *f* and *od* and between *od* and *car*. Thus the high temperature treatment increases both normal and unequal crossover frequency in the chromosome region studied.

If the unequals are produced by gonial crossing over this should be indicated by the presence of clusters. Although the offspring is not separated on individual mothers the result can be tested against the Poisson distribution according to the incidence of none, one, two or more cases of unequal crossing over among the offspring from each batch of 5 females. The following distributions were found:

	number of unequal cross overs			
	0	1	2	3
Controls	33	20	5	1
Treated	48	30	16	4

Both agree well with the expected distribution (χ^2=0.10 and 1.25 for 1 d. f.).

Another test possibility is to classify the cases of two or three unequals with regard to their being identical, complementary or different. Identical and complementary cases may have a common origin from the same exchange, if this is of the gonial type. The random distribution of pairs is: $^1/_4$ identical, $^1/_4$ complementary, $^2/_4$ others, and the actual distribution found:

	Ident.	Compl.	Others
Controls	2	1	2
Treated	2	7	7

thus there is no preponderance for the types with possible common origin.

When three unequals occur in the same offspring the probability is $^1/_4$ for combinations which may have a common origin and $^3/_4$ for combinations which must have at least two different origins. The actual distribution was: in the controls one case which could not be of common origin; in the treated one case which could and three cases which could not result from one gonial exchange.

Thus none of the distribution tests suggests the presence of clusters, and the experiments do not prove that the increased frequency of unequal crossovers is caused by gonial crossing over. It may be that the

material is too small to reveal a divergence from randomness of this kind, but possibly there may exist a real difference in the mode of action between X-rays and high temperature. Further experiments of the same type with X-rays as inducing agent are under way, which may provide the answer to this question.

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ON THE MUTAGENIC ACTION OF ETHYLENE OXIDE AND DI-EPOXYBUTANE IN BARLEY

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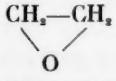
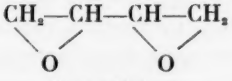
(Received April 15th, 1957).

INTRODUCTION

In our studies of the mutagenic action of different classes of chemicals (EHRENBERG *et al.*, 1956 a, b) a direct comparison of the effects of the simple and double epoxides, ethylene oxide and diepoxybutane was performed.



ethylene oxide



diepoxybutane

The investigation unveiled fundamental qualitative differences between the effects of the two compounds. Further, ethylene oxide turned out to be at least as efficient a mutagenic agent as the ionizing radiations, a fact of possible practical value, *e. g.*, for plant breeding by means of mutation. These circumstances will be treated in the present communication. In a later publication the material will be discussed on a broader basis, comparisons being made with other mutagenic chemicals.

The boiling point of ethylene oxide is 12° C. This makes it possible to treat the barley seeds with the gas itself as well as with water solutions. Since the diepoxybutane, on the other hand, boils at 140° C., the direct comparison of the two compounds has to be restricted to treatments with water solutions.

EXPERIMENTAL

Commercial pure ethylene oxide (Matheson Co., Inc.) and rac. 1,2: 3,4-diepoxybutane, kindly placed at our disposal by Dr. P. KOLLER, Chester Beatty Research Institute, London, were used. Due to the

extreme toxicity, especially of the latter compound, all handling and treatments had to be done under an effective ventilation.

Resting seeds, of the barley variety Bonus, as well as seeds presoaked for 24 hours on moist filter paper were immersed for two hours or more in solutions of the compounds, 30 ml solution being used for 150 seeds (volume ratio of solution to seeds=5 : 1). The suitable concentrations hade been determined in preceding tests in Petri dishes, involving measurements of germination frequency and growth rate of seedlings. All treatments were done at room temperature.

The effects of epoxides have turned out to be strongly temperature dependent (*cf.* also KIHLMAN, 1956). The field material of 1956 was treated at about 25°. In 1955 the temperature was about 20°. Ethylene oxide was found to be more effective in 1956. Direct comparison should therefore not be made between different experiments unless the temperature is well controlled. Another cause of poor agreement between different experiments has probably to be ascribed to the seeds passing, after 24 hours of soaking, a rather short sensitive stage, which is affected in some experiments, not in others.

After rinsing the seeds in running tap water, they were sown in the field. Analyses were done, according to standard methods (*cf.* EHRENBERG and NYBOM, 1954), of germination rate, survival at maturity, sterility, and mutation rate (chlorophyll mutations). Sterility is defined here as per cent sterile flowers per spike.

RESULTS AND DISCUSSION

Naturally a comparison between two chemicals with regard to their effectiveness cannot be based on the doses applied, in contrast to the case when effects of penetrating radiations are studied. Although the concentration in tissue could be measured, a dose estimation is made impossible by the discontinuous distribution of the compound. It was found in the preliminary germination tests (*cf.* also REVELL, 1953), however, that the biological effects of the epoxides studied are proportional to concentration and to duration of the treatment. In such a case a direct comparison is justified. The ratio of effectiveness can then be related to differences in diffusion rates, reaction rates, or specificity. In the following discussions the concentrations by weight are compared: they are practically proportional to the number of epoxy groups.

In the germination tests the degree of growth inhibition was found to be proportional to concentration. A 50 per cent decrease of plant height

TABLE 1. *Effectiveness of epoxides in barley seeds.*

Compound	Seed condition and time of treatment	I LD_{50} (at maturity) %	II conc. giving 1 % mutations %	III conc. giving 2 % sterility %	I/III	II/III
Ethylene oxide	Pre-soaked, 2 h	0.28	0.04	0.11	2.5	0.36
	Resting, 10 h	0.15	0.015	0.09	1.7	0.17
Diepoxybutane	Pre-soaked, 2 h	0.0008	0.0008	0.0004	2	2
	Resting, 2 h	0.0010	0.0006	0.0004	2.5	1.5

(control height: 80 mm) was obtained with 0.6 % ethylene oxide or 0.003 % diepoxybutane, when pre-soaked weeds were treated for 2 hours. The ratio of effectiveness thus amounts to 200 times. In these tests the diepoxybutane was decidedly more typically radiomimetic than the simple compound: germination did occur up to concentrations around 0.3 %, *i. e.*, 100 times larger than that causing 50 % growth inhibition. A similar picture is obtained after irradiation of resting seeds by X-rays, where 10^4 r cause about 50 % growth inhibition, whereas 10^6 r still permit germination in Petri dishes (EHRENBERG, 1955 a). This behaviour indicates that cell elongation is still possible, and that total growth inhibition, and later, death, do not occur until cell divisions become of critical importance. When seeds are treated with ethylene oxide, immediate seed mortality (*i. e.*, inability to germinate at all) occurs above 1 %, only 3 times the concentration of diepoxybutane causing the same effect. Obviously, the proximity of the concentration ranges giving radiomimetic and toxic effects, respectively, partly masks the radiomimetic properties of ethylene oxide. The small difference in effectiveness between the compounds with regard to incapacity of germination indicates that this effect is rather unspecifically dependent only on the concentration of epoxide groups.

Also in the field material a similar effect was found. In the case of diepoxybutane a few per cent germination still occurred at the highest concentration tested, 0.025 %, *i. e.*, about 30 times that giving 50 % survival at maturity (*cf.* Table 1). Above 0.0016 % all plants later died at the seedling stage.

The analysis of the field material is summarized in Figs. 1—2 and Table 1. It appears, that for both compounds *mutation rate* is practically rectilinear with concentration. At high concentrations, possibly, some elimination of mutations occurs. Relative to the lethal effects,

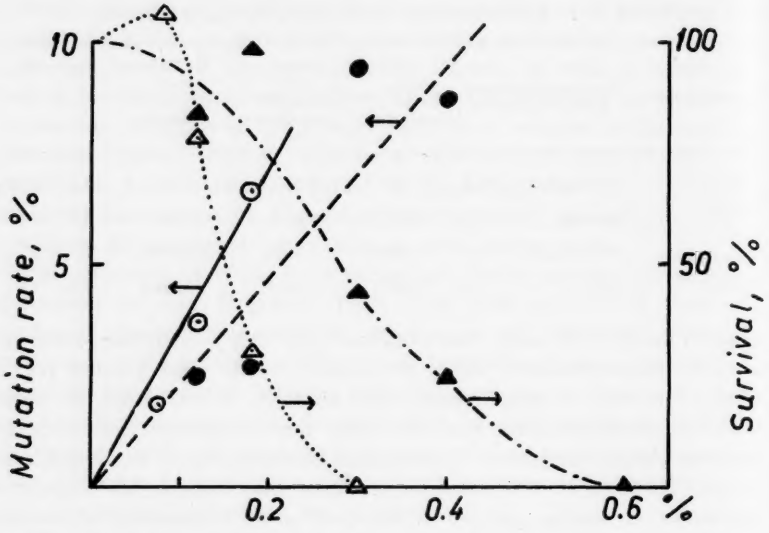


Fig. 1 a. Survival in the field (triangles, % of 150—300 seeds sown) and mutation rates after treatment with ethylene oxide. Empty symbols: resting seeds, 10 h; filled symbols: pre-soaked seeds, 2 h.

ethylene oxide is more specifically mutagenic, a fact that allows mutation rates around 10 % to be reached, in contrast to about 1 % for diepoxybutane.

The two compounds behave quite differently with regard to induced sterility. The sterility caused by ethylene oxide is an exponential function of the concentration, the exponent being of the order of 2, as could be expected if two chemical reactions of a certain kind (*e. g.*, two chromosome breaks, able to give a translocation) have to occur in order to produce a change, finally leading to sterility. The sterility curve thus resembles what is found when sparsely ionizing radiations are used (EHRENBERG, 1955 b). The double epoxide, on the other hand, causes a low rate of sterility almost proportional to concentration, with a possible elimination of sterility, at high concentrations with only a few plants surviving. There is, accordingly, some similarity of the sterility induced by neutrons, where one and the same ionizing particle — preferably a proton — is able to produce two breaks leading to a translocation. This similarity is, however, certainly superficial in

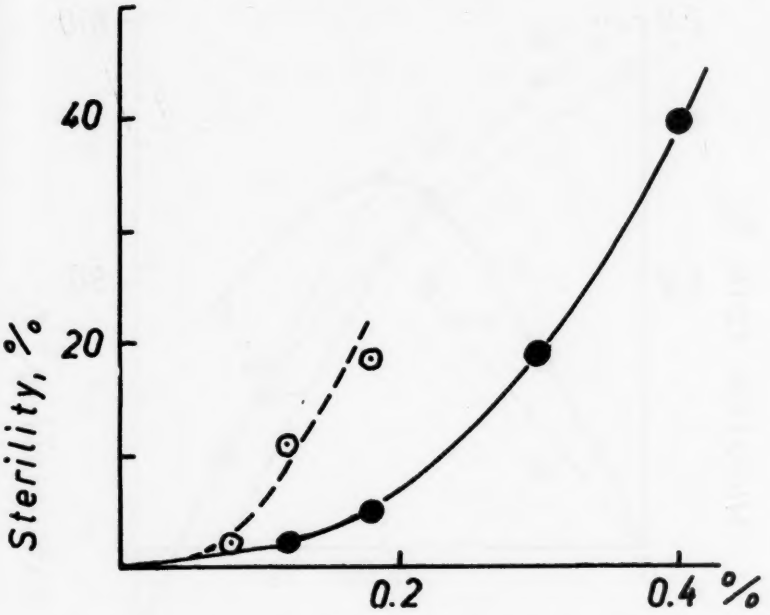


Fig. 1 b. Sterility in the material of Fig. 1 a.

character, since neutrons permit very high sterilities to be achieved before the material is killed (*cf.* EHRENBERG and NYBOM, 1954).

The sterility at low concentrations is proportional to the plant mortality. About twice the concentration of both compounds giving 2 % sterility is enough to kill half the number of plants. It seems therefore justified to assume that the decrease of plant survival has to be ascribed to initial chemical changes of the same kind as those causing sterility. For an elucidation of this point the study of the first mitotic cycle of the germinating seeds will be necessary.

The proportionality to concentration and duration of action, found for the different effects, indicates that the compounds react directly with the nuclear material (chromosomes, genes), not via effects on enzymes or other biochemical mediators. This explanation is supported by KIHLMAN's (1956) studies of the production of chromosome aberrations in *Vicia* root tips by di(2,3-epoxypropyl)ether: a simple influence

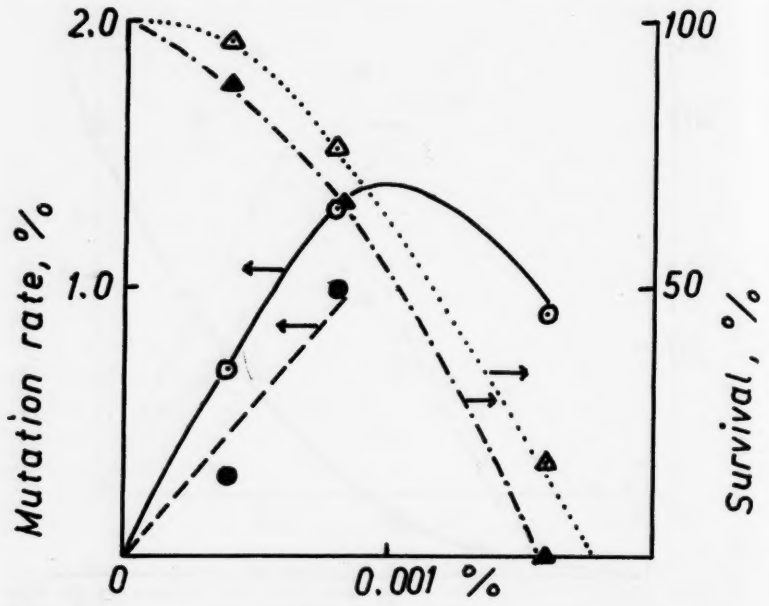


Fig. 2 a. Survival in the field (triangles, % of 150—300 seeds sown) and mutation rates after treatment with diepoxybutane. Empty symbols: resting seeds, 2 h; filled symbols: pre-soaked seeds, 2 h.

of temperature during treatment was found and, in addition, anoxia had no effect.

The outstanding efficiency of diepoxybutane in causing lethality and sterility (about 300 times that of ethylene oxide) might be ascribed to its double epoxide character, permitting the formation of bridges within or between macromolecules, *e. g.*, nucleic acids (ALEXANDER, 1952; BACQ and ALEXANDER, 1955). The diepoxide is certainly also the most active mutagenic agent of the two, but in this respect it is superior to ethylene oxide by a factor of only about 50 — compare KÖLMARK and WESTERGAARD (1953), who found the two compounds about equally mutagenic in *Neurospora.*

Relative to other effects the simple ethylene oxide is about 6 times more specifically mutagenic than diepoxybutane (*cf.* Table 1, last column). The induction of a gene mutation is obviously not as dependent on alkylation at two adjacent points as is the production of steri-

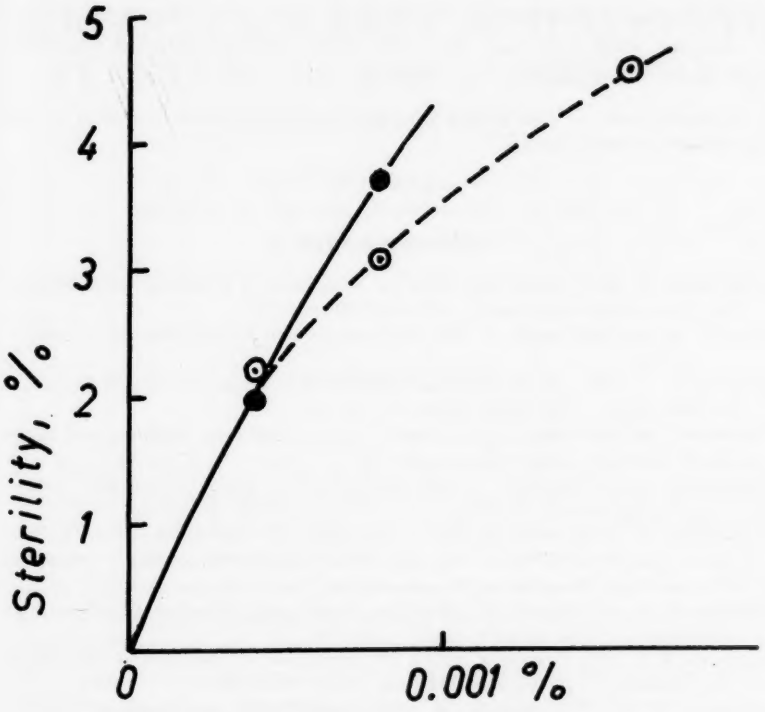


Fig. 2 b. Sterility in the material of Fig. 2 a.

lity or decrease of survival: the present experiments indicate that in many cases a single alkylation will be sufficient. The possibility by chemical means to differentiate between mutagenicity and sterilizing activity is thus shown once more (*cf.* EHRENBERG *et. al.*, 1956 a).

As regards the types of chlorophyll deficient mutants obtained, a rough classification reveals no difference between the compounds:

Compound	Relative frequencies of chlorophyll mutation types %				Total No. of cases
	albina	viridis	xantha	others	
Ethylene oxide	35.6	50.0	2.9	11.5	104
Diepoxybutane	29.4	52.9	5.9	11.8	17

About the same distribution of mutation types as produced by X-rays is obtained with a surplus of viridis types characteristic of relatively high doses (EHRENBERG *et al.*, 1956 b).

Acknowledgment. — The present investigation was supported financially by the Agricultural Research Council.

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MEIOSIS AND POLLEN MITOSIS IN RYE PLANTS WITH MANY ACCESSORY CHROMOSOMES

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I. INTRODUCTION

THE material investigated is based on the cultures of Professor ARNE MÜNTZING. Thanks to him, accessory chromosomes (B chromosomes) in rye are better known than in any other species. The most common type, at first called standard fragment, is here called standard accessory. It is smaller than ordinary rye chromosomes (A chromosomes) and has subterminal centromere. Certain other types exist but a derivation from the standard accessory has in most cases been proved by MÜNTZING. Instances are: a large and a small iso-chromosome, a chromosome with a terminal deficiency and a minute chromosome with a deleted centromere (MÜNTZING, 1944; MÜNTZING and LIMA-DE-FARIA, 1953, LIMA-DE-FARIA, 1954).

In natural rye populations one usually finds only two accessory chromosomes in a plant, more rarely four, but by means of crosses between plants of the Wasa II variety with four accessories MÜNTZING (1954) has obtained plants with 5—9, and as many as 10 after crossings between plants with 6 accessories. Such rye plants are highly or completely sterile and their vigour is reduced. Meiosis in plants with one and two accessories has been investigated by MÜNTZING. In the Wasa II variety a small bivalent was observed in 90 per cent of the pmc, but in Östgöta gråråg only in 30 per cent, the majority of pmc here having two small univalents. Östgöta gråråg plants with one large iso and two standard accessories showed random pairing between these chromosomes, a heterobivalent being twice as frequent as a bivalent of two standard accessories. But in plants with this large Östgöta gråråg chromosome and two standard accessories of the Wasa II variety, heterobivalents were present in only 2 per cent of the cells. Thus there is a

marked reduction of pairing when accessories of these two varieties are brought together (MÜNTZING, 1949). Weakened pairing at diakinesis and first metaphase is a property of most accessory chromosomes, full bivalent formation being more exceptional.

Here observations on the pairing in plants with six and eight standard accessories will be described. The first pollen mitosis has also been studied not only in plants with many, but also with four, accessories. More occasional observations on the second pollen mitosis and on the embryo sac have also been made.

All fixed material was imbedded and sectioned before staining.

II. MEIOSIS

The plants belonged to the Wasa II variety which has good pairing of accessories. Three individuals had eight accessories but additional observations were made on two plants with six. Earlier stages than diakinesis were not investigated. In Östgöta gråråg plants with two and four standard accessories, it is known that the latter form bivalents at pachytene and early diplotene though the partners in most bivalents are separated at diakinesis as univalents.

The diakinesis stage was often less distinct than desirable, the chromosomes often adhering. However, in plants with eight accessories eleven bivalents were observed, four of them small. This ideal pairing is rather frequent. More often, however, there were small univalents, most often two (Fig. 2), then four, but rarely a higher number.

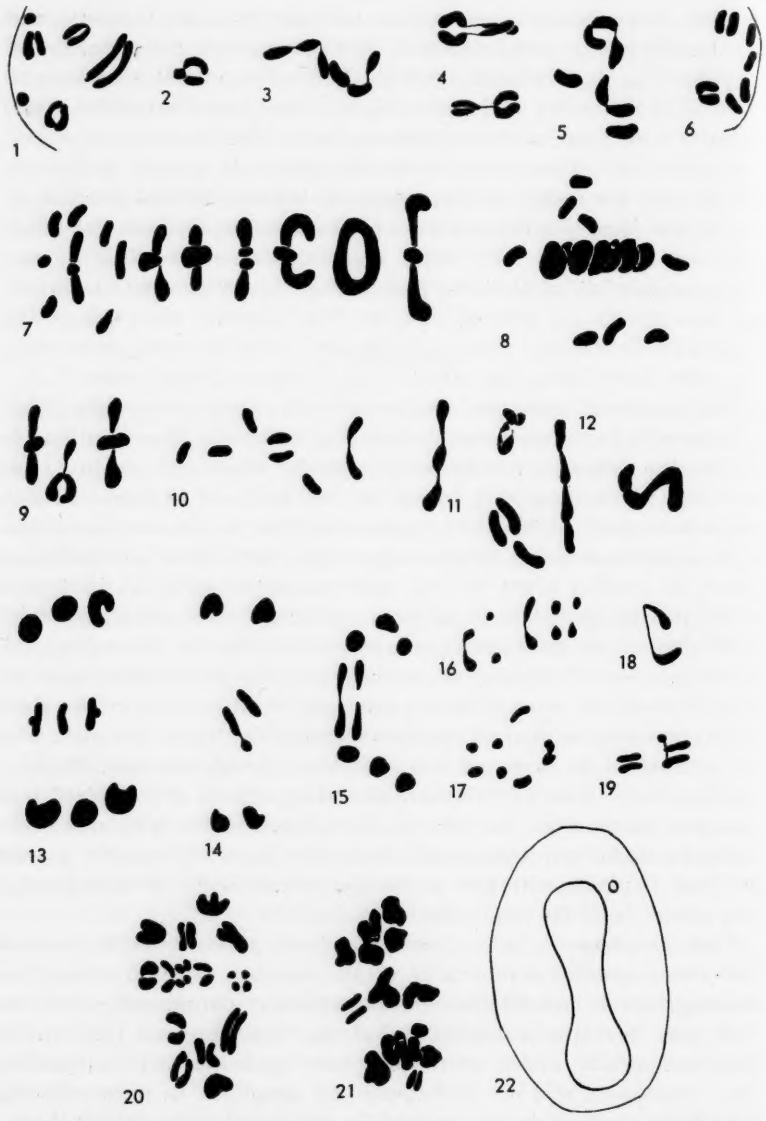
In contradistinction to large bivalents the small ones have no interstitial chiasma, an exception being shown in Fig. 2. The small bivalent may have two terminal chiasmata, but very often it has only one; the two partners are then in a row or lie parallel. Notwithstanding the high number of accessories multivalents were only rarely observed. Instances of trivalents and quadrivalents from different nuclei are shown in Figs. 3 and 4. Only very rarely was a triple chiasma observed. In a number of nuclei a row of five to seven accessories was met with (Fig. 5); this could be a multivalent, but the connections between the chromosomes were generally incomplete, and the interpretation multivalent is not sure; it may be rows of univalents and open bivalents.

After the disappearance of the nuclear membrane the univalents have a peripheral position in relation to the group of large and small bivalents. Most univalents are thus outside the plate which is composed

of seven large bivalents, a varying number of small bivalents and occasionally one or two univalents. The latter are in the periphery of the plate (Fig. 8). If several small bivalents were present, they were to be found in the centre of the plate, that is, they have the position usual in plates with large and small chromosomes. Multivalents are absent from most cells. Trivalents and quadrivalents are shown in Figs. 11 and 12; they are mostly a chain placed in the longitudinal direction of the spindle. Most small bivalents have one terminal chiasma. On a few occasions a trivalent with triple chiasma was observed. This was always somewhat outside the plate, being nearer the pole to which two centromeres are directed (Fig. 9). This confirms observations and theories by ÖSTERGREN (1951) of attraction forces between centromeres and poles, these being also effective in accessory chromosomes.

The number of univalent accessories varies from nil to eight. They are generally more numerous than during diakinesis. One plant had in 87 pmc the following number of univalents: eight in 3, six in 7, five in 1, four in 39, three in 3, two in 24, one in 3 and in 7 pmc no univalent was observed. The odd numbers are due to trivalent formation, or to a univalent being hidden among the chromosomes in the plate. Counts in another plant showed eight univalents in 1, six in 4, four in 20, two in 26, while in 13 pmc univalents were lacking; a third count showed six univalents in 2, four in 55, two in 40 and no univalent in 7 cells. In the latter counts pmc with odd numbers were excluded. Thus the most common numbers of univalents in the three counts was two and four. A curious exception was found in a plant with six accessories. In some anthers complete pairing was very frequent, namely, in 137 pmc of 182 counted. Other anthers of this plant had, however, more often univalents. The plants with eight accessories showed a higher percentage univalents than expected from the pairing in Wasa II plants with two accessories, which had two univalents in only about $^1/_{10}$ of the pmc (MÜNTZING, *l. c.*).

First anaphase is in the absence of univalents perfectly normal. Univalents usually divide later at the equator. Fig. 15 shows two dividing univalents while three whole accessory chromosomes are near each pole. Sometimes divided univalents were observed high on the anaphase spindle at the pole. They have probably had this position since metaphase and not undergone any anaphase. In most cases the univalents on the spindle move to the equatorial plate, taking there a peripheral position, dividing during the anaphase movements of the previously paired chromosomes. As many as six accessories have been



Figs. 1—22. Meiosis in plants with eight accessory chromosomes. — Figs. 1—6, accessories at diakinesis. Fig. 1, $3_{II}+2_{I}$. — Fig. 2, a small bivalent with interstitial chiasma. — Fig. 3, 2 trivalents in the same nucleus. — Fig. 4, quadrivalents from different

observed dividing at the equator. Rarely do they divide on the half-spindle. ÖSTERGREN and VIGFUSSON (1953) have studied the movements of large iso-chromosomes on the spindle and point out that they move on the surface of the spindle as ordinary univalents. Splitting of the univalent often begins in the long arm. The accessory may then look like a small bivalent, with the short arm in the plate, the chromatids of the long arm being directed towards the poles (Fig. 13). Often the separated chromatids are parallel (Fig. 19), only somewhat later are the centromeres directed towards the poles, the pulling forces having then set in. Fig. 18 shows the dividing of a univalent which was on the spindle midway between equator and a polar group, one end showing traces of a certain stickiness. Lagging chromosomes in similar positions may show indications of fragmentation; the two arms may lie apart but are often united through a thread (Fig. 14). This is similar to mis-division of the centromere of the accessory, a process which may result in iso-chromosomes. Notwithstanding the frequent lagging observed, it is only more rarely that a chromatid or part of a chromatid is left outside the telophase nuclei.

Development was often delayed or interrupted in pmc with an abnormal position in the centre of the loculus, lacking contact with tapetal cells. In cases where the chromosomes were developed, they were unusually long, and ordinary chromosomes showed weakened pairing. One loculus contained a syncytium, 29 pmc having united. The nuclei showed late diakinesis; a few were free, but most of them had fused forming a very long, irregular nucleus with separate chromosome groups. Also in the lower part of the anthers meiosis may be delayed with sometimes long chromosomes and large univalents. Presumably the activity of the tapetal cells is lowered. Larger irregularities predominantly occur in such regions. Instances of this are a bridge between telophase nuclei, sometimes persistent after the formation of the wall; no fragment was observed, the bridge being caused by stickiness. Anaphase was sometimes faulty and may result in the

nuclei. — Figs. 5—6, dubious multivalents. — Figs. 7—12, accessories at first metaphase. Fig. 7, $8_{II}+6_{I}$. — Fig. 8, 8_{I}. — Fig. 9, trivalents between two ordinary bivalents (*in situ*). — Fig. 10, $2_{II}+4_{I}$. — Fig. 11, trivalents from different pmc. — Fig. 12, quadrivalents from different pmc. — Figs. 13—22, accessories at anaphase. — Fig. 13, lagging in the plate. — Fig. 14, fragmentation. — Fig. 15, two univalents dividing, in three bivalents the partners have separated. — Figs. 16—17, dividing univalents. — Fig. 18, univalent dividing on the half-spindle. — Fig. 19, univalents dividing at the equator. — Figs. 20—21, very disturbed anaphases, not all chromosomes delineated. — Fig. 22, restitution nucleus and a very small micronucleus.

forming of a restitution nucleus (Fig. 22) or in rare cases of three telophase nuclei. The chromosomes in such cases move very slowly, their movements being interrupted before they are far apart (Fig. 20). The chromosomes split and the forming of nuclear membranes begins. Three chromosome groups, one of them lagging at the equator, may result in three telophase nuclei (Fig. 21).

The formation of restitution nuclei in connection with a high number of accessory chromosomes at meiosis was observed in *Festuca pratensis* (BOSEMARK, 1954); this was caused by the slow division of many lagging accessory chromosomes, but in some cases the cause was physiological. The cases observed in rye were of the latter kind.

The second metaphase shows regular plates in both dyad cells. Thus the chromatids from univalents divided at first anaphase are arranged in the plate. The subsequent anaphase was in most cases regular but with chromatids from the first anaphase lagging. Thus centromeres of accessory univalents divide once during meiosis though a second division is perhaps not ruled out in view of the mostly low number of laggers. Cases with one cell with diploid chromosome number leading to pollen diads were observed. Loculi with strongly disturbed divisions may have one to many bridges in sometimes nearly 100 per cent of the pmc; the chromosomes were apparently very sticky here. A further anomaly was seen in that wall formation may have failed during first telophase. Elimination is much less frequent than lagging also in this division.

III. POLLEN MITOSIS

A curious disturbance of the first pollen mitosis was often observed in some plants of Wasa II having six standard accessories (HÅKANSSON, 1948). It was also found in a Östgöta gråråg plant with four accessories, though less frequently. The chromosomes do not form a metaphase plate but are spread in different ways in the pollen cell. They split, often forming so-called ski pairs. A spindle is present but anaphase, if occurring, is fragmentary, and telophase often showed supernumerary nuclei or a restitution nucleus. This curious disturbance also occurred on the female side, to which a large restitution nucleus or more or less numerous nuclei of different sizes bore witness.

The division was compared with c-mitosis (stathmokinesis), but was mostly more similar to so-called partial c-mitosis (ÖSTERGREN, merostathmokinesis of GAVAUDAN, 1947). Such disturbances had been observed after treatment with more dilute solutions with colchicine, these

only causing a partial inactivation of the spindle (ÖSTERGREN, 1950). GAVAUDAN discerns three stages: tropokinesis, where only the direction of the spindle is changed (»Leur obtention nécessite les doses les plus faibles des substances les plus actives»), merostathmokinesis (formation of several nuclei) and stathmokinesis. The Östgöta gråråg plant which had pollen cells with one, two or three accessories showed upset of mitosis only in cells with three. An increased effect with higher number of accessories is evident here.

Some plants with seven, eight and nine accessories have now been investigated. In addition, results from investigation of more plants with four and six accessories are reported.

All plants showed in different frequencies, (1) normal first mitosis, as a rule with non-disjunction of the accessory chromosomes, (2) minor irregularities; the spindle is bipolar and two telophase nuclei are formed, this being comparable with tropokinesis, (3) upset mitosis, comparable with merostathmokinesis and more rarely, stathmokinesis, the spindle is not bipolar, more than two telophase nuclei and one restitution nucleus, respectively, being formed.

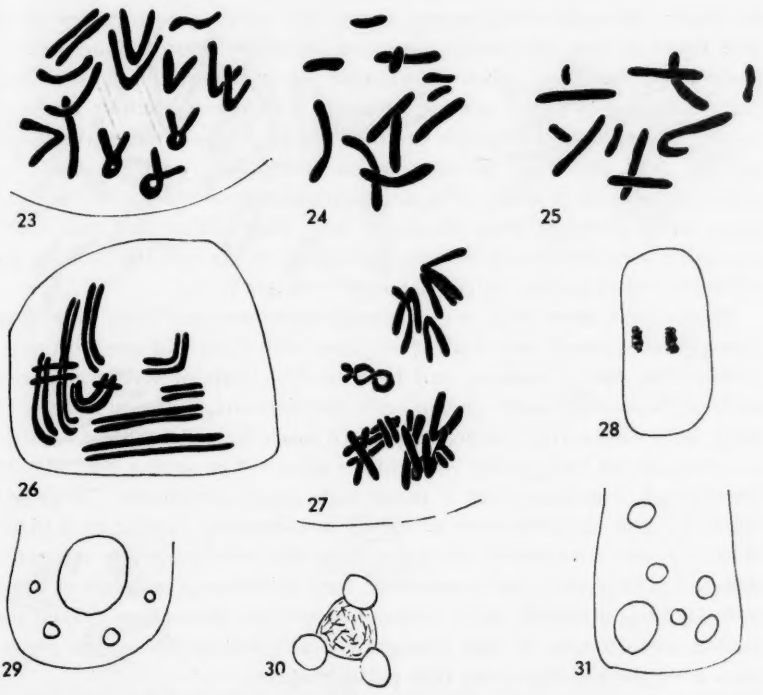
The minor irregularities are of a very different kind. Often they concern only the direction of the spindle. A transverse spindle leading to two free nuclei of equal size and structure is frequent. This has often been observed and described by many authors, and is therefore not surprising here. An oblique spindle leading to formation of an oblique wall was rare. In plants with a high number of accessories the pollen nucleus had sometimes not moved to its place near the end of the cell. Very large generative cells may thus be formed or the spindle may be directed against a side wall (Fig. 28), where in rare cases a generative cell was seen. To this instability the increased size of such pollen cells perhaps contributes, for they are rather broad.

The bipolar spindle may have an incomplete plate. One or two chromosomes may lie on the half-spindles, possibly later, before anaphase, taking their place at the equator. More irregular are cases with one or more chromosomes lying outside the spindle, splitting in the cytoplasm. Such chromosomes may also be observed between the generative spindle pole and the wall; also accessories may have such a place, thus perhaps being included in the generative nucleus without anaphase movement. In many metaphase spindles the chromosome arms are not in the equatorial plane but are directed towards the poles. The centromeres may have a position in the plate but the connection between spindle fibre and centromere often seems to be faulty. In plants with many

accessories anaphase in a bipolar spindle is fairly often irregular, with bridge formation, lagging of chromosomes and elimination.

The frequency of upset mitosis in *plants with four standard accessories* varied. In all, eight plants of Östgöta gråråg, two of Wasa II, one of a variety from Spain and one plant from Afghanistan have been studied. In certain Östgöta gråråg plants the stages of spread-out chromosomes, splitting and formation of more than two telophase nuclei were not unfrequently observed. In other plants only the first of these stages which is by far the longest one and may end in a normal metaphase, was observed. Three accessories could in favourable cases be counted among spread-out chromosomes. Clearly the frequency of upset mitosis was different in different Östgöta gråråg plants. Less variable was pollen mitosis in Wasa II where the large majority of pollen cells had two accessories and thus mitosis was more rarely upset. In the Spanish rye mitotic upset was fairly frequent. The different frequencies observed were probably mainly due to the course of meiosis, which in Östgöta gråråg must lead to a variable number of accessories in the pollen cells. Fig. 23 shows ten chromosomes. Here the A chromosomes have divided, the centromeres are unipolar-directed, but anaphase pulling has hardly begun. This is an instance of unipolar spindle. Upset mitosis was more frequent in *plants with six accessories*. This was particularly evident in Wasa II where most pollen cells had three accessories, this relative constancy being a result of the regular pairing at meiosis. In the Östgöta gråråg plants the frequency was considerably lower and the difference between pollen mitosis in plants with four and six accesories was less distinct here. In the rye from Spain the difference in mitosis between plants with four and six accessories was considerable. The variable mitosis was earlier closely described. The chromosomes are often greatly spread-out, sometimes having peripheral positions in the cell, recalling cases described as distributed c-mitoses (compare ÖSTERGREN, 1950). In Östgöta gråråg a few pollen cells with four or five accessories were also observed (Fig. 24).

A plant with three standard and three small accessory chromosomes of a type that has not been studied previously, once detected by ÖSTERGREN, showed a high frequency of upset divisions. The prophase nucleus often had unusual positions in the cell, which often was broad. Regular formation of generative cells was observed but more frequently mitosis was upset. Fig. 27 shows a curious case, in which the chromosomes at right undergo normal anaphase while at left there is no spindle action. Lagging of accessories on the spindle was rarely observed but



Figs. 23—31. Upset first pollen mitosis in plants with 4—6 accessories. Fig. 23, Östgöta gråråg with 4 accessories; unipolar spindle with splitting chromosomes, 3 accessories. — Fig. 24, Östgöta gråråg with 6 accessories, the pollen cell had five (four?) accessories. — Figs. 25—27, plant with 3 standard and 3 small accessories. Fig. 25, 2 st. and 2 small. — Fig. 26, 2 st. and 1 s., all chromosomes except the small one are divided. — Fig. 27, anaphase, incomplete bipolar spindle, the small accessory seems to have undergone non-disjunction. — Fig. 28, normal telophase. — Figs. 29—31, after telophase. Fig. 29, restitution nucleus and four micronuclei. — Fig. 30, three nuclei from a tripolar spindle, which still persists. — Fig. 31, six nuclei of different size.

non-disjunction surely occurs, the two anaphase groups being of different size. But as it is difficult to observe the behaviour of accessories when their anaphase is not delayed, the normal behaviour of the small accessory was not quite clear. However, some evidence of non-disjunction was obtained (Fig. 27).

The small accessories may be observed after the disappearance of the nuclear membrane, and Fig. 25 shows two standard and two small accessories. Fig. 26 shows a case with two standard and one small

accessory. Here all chromosomes except the small accessory are split. The latter is less frequently observed than the standard accessory; probably it is more often eliminated at meiosis. Telophase often gave evidence of upset mitosis, showing a lobed restitution nucleus, several supernumerary nuclei or three nuclei formed from a tripolar spindle (Fig. 30). The division is on the whole rather similar to pollen mitosis in a plant with six standard accessories, upset mitosis being more frequent than in plants with four. Thus also this short accessory seems to have a detrimental effect on the spindle, perhaps an influence equal to that of the standard accessory.

Plants with more than six accessory chromosomes have also been investigated, namely, two with seven, four with eight and one with nine. Elimination during meiosis had been modest, four or five accessories were often counted in the pollen cells. An increasing but low number of large cells with a larger nucleus is present when the number of accessories is enlarged, but only rarely was mitosis observed in such a cell. Fig. 32 shows such a mitosis from a plant with seven accessories. There are about 21 split chromosomes which lie in a manner similar to a plate. However, the centromeres of the chromatids were generally not pole-directed as in normal meta-anaphase; here we have an instance of upset mitosis. Thus a diploid set of chromosomes is no protection against the mitotic disturbances as was thought a possibility in the earlier paper: they are a peculiarity of the first pollen mitosis.

The young haploid pollen grains are often considerably larger than in plants which have no accessory chromosomes. Striking is the increase in breadth, the cells often becoming rather isodiametric. MÜNTZING and AKDIK (1948) have, by measuring stomata, shown that »standard fragments as well as large isofragments cause an increase in cell size». This is valid for pollen cells, too.

Also plants with eight and nine accessories have many pollen grains with a generative cell. Particularly in the plant with nine accessories had wall formation, however, often failed. Fig. 33 shows eleven split chromosomes, most of which rather distinctly form a plate; but no anaphase movement has begun. There is thus a spindle here. Upset pollen mitosis is common in plants with eight accessories but generally the chromosomes were less spread-out than in plants with six. Many cases were, however, very similar to the one in Fig. 34 with five accessories. But in a number of cells a spherical chromosome group was observed, in which all chromosomes were doubled. The chromosomes maintained in such cases their position from prophase to telophase.

Figs. 32—44, upset pollen mitosis in plants with 7—9 accessories. — Fig. 32, from an unreduced pollen cell, 14 ordinary and 7 accessory chromosomes. — Fig. 33, four accessories of different appearance, most chromosomes here form a plate. — Fig. 34, five accessories. — Fig. 35, divided chromosomes forming several groups. — Fig. 36, stage approaching telophase. — Fig. 37, somewhat later, spiralization of chromosomes. — Fig. 38, lobed restitution nucleus. — Fig. 39, elimination after a bipolar anaphase. — Fig. 40, a curious bent spindle between daughter nuclei. — Figs. 41—43, restitution nuclei. — Fig. 45, the second mitosis, early telophase. — Fig. 46, tube nucleus and sperms. — Fig. 47, irregular embryo sac from a plant with nine accessories.

In the plant with nine accessories such spherical groups were, it seemed, more frequent and their change to a spherical restitution nucleus was clear. Some of the accessory chromosomes may be outside the sphere. A complete suppression of the spindle has apparently occurred in such cases, and this was scarcely observed in plants with six accessory chromosomes. Particularly the plant with nine chromosomes generally showed weaker spindle formation than any plant with six accessories.

In certain species with a high number of accessories division of the tube nucleus has been observed to divide immediately after the formation of the generative cell. In rye this is a rare phenomenon, observed a few times in the plants now discussed. No second pollen cell was found, however. In a pollen grain where no cell had been formed and therefore containing two free nuclei, prophase was observed in both. Some peculiar restitution nuclei are to be seen in Figs. 41—44.

In these plants with a high number of accessory chromosomes pollen mitosis often failed, the nucleus remaining in the resting stage. A plant with four standard and four small accessories showed in some observed cases among outspread chromosomes: 3 standard and 3 small, 2 standard and 2 small, 3 or 4 standard, but also 3 small accessories. Upset mitosis was frequent but a regular generative cell was also often observed.

The *second pollen mitosis* was studied in some plants with four or six accessories. The spindle is in this division long and slender, the chromosomes therefore being close together in the plate. At late anaphase the spindle often becomes bent, in which cases the sperms take their position partly on each side of the tube nucleus (Figs. 45 and 46). Occasionally a lagging chromosomes was observed during anaphase but it did not as a rule result in elimination. Plants with six accessories formed sperms only in a minority of the pollen grains, either because a generative cell had not been formed, or because it had degenerated. Any division of a restitution nucleus was not observed.

Embryo sac development was the object of some study. Clear instances of the first mitosis were not observed, but supernumerary nuclei indicated that this division had often been irregular in plants with six accessories, also in the plant with 3 standard and 3 small accessories. In plants with eight accessories development in the ovule was much delayed, the embryo sac being often seen degenerating at the uninucleate stage. However, one may find ovules containing a seemingly normal embryo sac. Fig. 47 shows an abnormal embryo sac from the plant with nine standard accessories.

IV. DISCUSSION

Rye plants with many accessory chromosomes are highly or completely sterile; they have also reduced vigour. Meiotic irregularities do not contribute much to this sterility; meiosis is rather regular, the accessories having no influence on the pairing of the ordinary chromosomes. So far no accessories have been found in Gramineae which show complete asyndesis, as is frequent among dicotyledons, *e. g.*, in *Centaurea scabiosa* (FRÖST, 1956). The pairing is different in different rye varieties, but multivalents are rare even in rye plants with eight accessory chromosomes. Also *Poa alpina* meiosis very rarely shows any multivalent accessory chromosomes, notwithstanding their good pairing (MÜNTZING, 1948, HÅKANSSON, 1954). In other grasses, however, multivalents are formed, in *Anthoxanthum aristatum* in equal frequency with A chromosomes (ÖSTERGREN, 1947), rather frequently in *Zea mays* (BLACKWOOD, 1957), in *Festuca pratensis*, *Phleum phleoides* and some other grasses (BOSEMARK, 1956 and unpublished). Possibly it is merely a coincidence that only such accessories as are not clearly heterochromatic form few multivalents.

In *Phleum phleoides* and some other grasses BOSEMARK has observed neocentromeric activity of the ends of accessory chromatids moving to the poles during first anaphase. He has connected this behaviour with the heterochromasy of the accessory chromosomes. This was not observed in rye; which is in agreement with the view of BOSEMARK. Univalent accessory chromosomes divide at first anaphase, the same being reported from other grasses. But in *Anthoxanthum aristatum* they go undivided to the pole (ÖSTERGREN), the same being valid for *Poa alpina*, here MÜNTZING stresses a difference in comparison with the behaviour of A-univalents. Such a difference consequently does not exist in most other cases. Several authors, *e. g.*, BLACKWOOD concerning *Zea*, mention that elimination of accessory chromosomes is much less frequent than could be expected from lagging univalents: the same is valid for rye.

An important cause of the sterility of rye plants with many accessories was the upset of the first mitosis in microspores and macrospores, that is, the division that regularly shows non-disjunction of standard accessories. The spindle is incomplete, and a regular metaphase and anaphase do not occur. The distribution of the chromosomes of telophase is very different and the three forms described and defined by ÖSTERGREN (1950) are observed, namely, (1) multiple distributions corresponding to partial c-mitosis or merostathmokinesis, (2) apolar, corres-

ponding to complete spindle inactivation (full c-mitosis or stathmokinesis), one »diploid» or several aneuploid pollen nuclei being formed, and (3) the unipolar distribution from an unipolar spindle. The latter spindle had its only pole in such observed cases as the generative pole, which is directed against the wall, the chromosomes being close to the wall. Evidently this pole is generally favoured, which is reflected in the non-disjunction of accessories to this pole in grasses. Curious cases with a bipolar and an apolar part of the spindle (p. 610) or with anaphase separations in only some of the chromosomes in parts of an undifferentiated spindle were observed (*cf.* HÅKANSSON, 1948). All these irregularities lead to an interruption of the development of the pollen cell before formation of sperms.

The appearance of the chromosomes is different from typical c-mitosis: in the latter, the chromosomes are supernormally contracted and after splitting look like diplochromosomes because the division of the centromere is delayed. Here the chromosomes are mostly not more but rather less contracted than in normal divisions and do not form diplochromosomes, because the centromeres divide at about the same time or slightly after the chromosome arms. The chromatids then appear as so-called ski pairs, well-known from many cases of disturbed mitosis. Exceptionally, however, the pollen cell had short diplochromosomes. Sometimes the whole chromosome except the ends was split, indicating that the split here begins at the centromere region.

These disturbances must result in sterility. Even if a restitution nucleus were able to divide, it seems improbable that such a pollen grain could effect pollination in view of the absence of a tube nucleus; this should impede the formation of a normal pollen tube.

There are no other instances of a similar disturbance of pollen mitosis owing to accessory chromosomes. It is probable that this division generally is particularly sensitive to disturbing influences; the fact is, that changes in spindle positions and directions are fairly often described. Cases of larger disturbances in the pollen cell in connection with a higher number of accessory chromosomes are known from *Sorghum purpureo-sericeum* (DARLINGTON and THOMAS, 1941) and *Festuca pratensis* (BOSEMARK, 1954). In the former case the pollen nucleus, immediately after the generative cell has been formed, divides one or more times, forming new generative cells. During these extra divisions the accessories undergo non-disjunction to the generative pole, which as it seems did not occur at the first division. Thus we have a repetition of the first mitosis, non-disjunction not occurring at the first. In *Festuca*

pratensis with many accessories the latter as well as the ordinary chromosomes give evidence of a pronounced stickiness, all chromosomes forming bridges. An influence of accessory chromosomes on the other chromosomes though of a different kind and in another place in the life cycle was described by RUTISHAUSER (1956) in *Trillium grandiflorum*, where in the endosperm there is spontaneous chromosome breakage in a low frequency, 0.80 per cent, and where the presence of 1—3 accessories increases the breakage to 1.3 per cent. The accessories are small and heterochromatic chromosomes which have no influence on seed fertility, nor was the influence on endosperm mitosis cumulative, one and three of them having the same effect.

In rye the influence of standard accessories on the first spindle in the spores is cumulative and a comparison was made with the effect of increased concentrations of c-mitotic substances, as described by various authors (GAVAUDAN, 1947, ÖSTERGREN, 1950) and studied on living material by GAULDEN and CARLSON (1951). Theories to explain this spindle inactivation involve the destruction of the structure of the spindle because the active substances prevent the molecular orientation necessary for a normal spindle structure. ÖSTERGREN (1944), accepting the thought of BERNAL that the spindle was a tactoid, assumed that c-mitotic substances had an affinity to the protein molecules, becoming associated with the fibrous spindle molecules and causing them to fold and thus destroy the tactoid.

In the rye case discussed here, a chemical interpretation is not possible. The spindle defect is caused by too many accessories in relation to the ordinary chromosome, this perhaps being a result of their insufficient contribution to spindle formation. It is generally thought that the spindle is for the greater part formed from the nucleus, that is, from the caryolymph and the chromosomes. Particularly fibres uniting centromeres and poles, and causing the metaphase equilibrium and the traction of the chromatids to the poles, are formed from the chromosomes, alone or together with fibres from the pole (see SCHRADER, 1954). The centromeres seem to be the centres of substances or organizing forces that form the spindle; and they are thus the most important organizing factor in spindle formation. A small but complete spindle may be formed from only one chromosome as is demonstrated by the division of micronuclei after disturbed first anaphase in meiosis of species hybrids. The efficiency of a spindle must conform to the number of chromosomes it contains but because each chromosome gives a contribution, this is always achieved. Each of the chromosomes must con-

tribute to the whole spindle; only in some exceptional cases in Hemiptera do the chromosomes form separate spindles. It may be that certain accessory chromosomes give an inadequate contribution to the spindle. With one or two accessories a normal pollen mitosis with a bipolar spindle occurs, but with three or more the burden of three or more deficient chromosomes may be too large, whereby the spindle organization becomes delayed and more or less fails.

The position of accessory rye bivalents in the centre of the plate at first metaphase and of accessory Y-trivalents nearer one pole show a normal centromere. The careful investigations of MÜNTZING (1948) on the position of rye accessories in root plates also gave no evidence of weak centromere. Non-disjunction at first pollen mitosis seems, at least, in rye, to be caused not by a centromere peculiarity but rather by a stickiness of a segment of the long arm. However, the fact that the centromere of the accessory behaves normally in a functional spindle, which has been formed from all chromosomes, is not in conflict with the idea that this same accessory has given an insufficient contribution to the organization of this spindle.

To the great sterility of rye plants with many accessories other factors of a more diplontic nature also contribute.

The accessory chromosomes in rye show many peculiarities but, for instance, the most striking, non-disjunction at the first pollen mitosis, is common to the accessories in most other grasses. BOSEMARK (1956), who has investigated the cytogenetics and other aspects of accessories in several grass genera, opens wide perspectives in writing (p. 464) »The cytological studies have revealed in practically all respects a striking similarity between the accessory chromosomes in *Phleum phleoides* and such chromosomes in other grasses like maize, rye, *Anthoxanthum* and *Festuca*. This and the study of still other grasses, with accessory chromosomes, (BOSEMARK, unpublished), show that the accessory chromosomes in the family Gramineae belong to a fairly homogeneous group, although in each species the behaviour and effects of accessories varies in minor details.» Exceptional traits of rye accessories are that they are not heterochromatic, though now LIMA-DE-FARIA (1948) has shown the presence of a subterminal knob in the long arm; it is the only case which has non-disjunction also on the female side as was shown genetically by MÜNTZING and cytologically by HÅKANSSON; it is also the only case with mitotic upset, but perhaps investigation of plants with a higher number of accessories may give examples of this anomaly in other species.

Accessory chromosomes have now been found in many plants but a genetical investigation has in most cases not been performed. The accessory element of the idiogram has a rather different appearance and the properties of its chromosomes may also differ. A large class of accessories consists of very small chromosomes, often heterochromatic and in most cases occurring in fairly high numbers within the plant. There are also instances of only a few small chromosomes. Such chromosomes have been found in many dicotyledons but also in some monocotyledons. The most thorough investigation of such chromosomes has been made by FRÖST on *Centaurea scabiosa*. A second class which is common in di- and monocotyledons is larger chromosomes, sometimes approaching the size of ordinary chromosomes, and occurring in a low numbers within the plant. They may or may not be heterochromatic. There are certain types of accessories which seem to form special cases. One such is found in the accessories of *Clarkia* (or *Godetia*) which seem to be modified ordinary chromosomes. A further such special, phylogenetically uniform group is the grass accessories which are the best investigated of all. Thanks to BOSEMARK their general occurrence within the family is clear. Through non-disjunction at pollen mitosis they have a mechanism by which to increase their number.

Concerning the role of accessories opinions diverge. There is agreement, however, on the injuriousness to the plant of a too high number and in rye they even destroy an important mitosis in such cases.

SUMMARY

In rye plants with eight accessories the latter may form four bivalents but the pairing is more often incomplete, two or four, rarely a higher number of univalents being observed. Multivalent formation is rare. Univalents divide at first anaphase. Occasionally a restitution nucleus is formed.

The first pollen mitosis is upset in increasing frequency with increased number of accessories. The presence of three or more accessories in the cell delays or prevents the forming of a bipolar spindle, possibly owing to a subnormal spindle-organizing activity of such chromosomes. In extreme cases there is no spindle.

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ABERRATIONS IN THE NUCLEOLAR CHROMOSOME OF INBRED RYE

II. SIZE VARIATION IN INBRED LINES AND POPULATION PLANTS

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I. INTRODUCTION

IN a previous publication (BOSE 1956) it was shown that certain types of aberrations could be detected in the nucleolar chromosomes of some inbred lines of rye. Since then, seven more inbred lines and some of their hybrids have been examined and similar aberrations have also been found in them. Details of this investigation have been presented here together with the results of an analysis of some fifty population plants. Such an analysis was considered necessary in order to trace the origin of these aberrations. It has been shown here that many of the aberrations known to be present in the inbred lines are also present in the population. It is considered most likely therefore that at least some of the types of aberrations in the inbred lines have come from the population after having become fixed through successive inbreeding. Their presence is regarded as an important piece of evidence in support of the hypothesis put forward by MÜNTZING (1938, 1945), that small cytological aberrations present in the population of cross fertilized plants may also play a role in bringing about inbreeding degeneration.

Definitions

It might be useful here to define the meaning of some of the more commonly occurring terms in this paper. These have been given below, with the approximate sense in which they have been used.

Chromosome. Unless otherwise stated, the word chromosome, has always been used in connection with the nucleolar chromosome. The nucleolar chromosome has been divided into three regions, the long arm region, the short arm region and the satellite region.

Long arm. This region extends from the distal end of the long arm to the kinetochore and is often referred to in the text by the letters L. A.

Short arm. The short arm has been divided into two regions: the short arm proper and the satellite. The term short arm as used in the text always refers to the region lying between the kinetochore and the nucleolar constriction region. It has been denoted in the text by the letters »A» or »a». The capital »A» being used in describing a large short arm and a small »a» in describing a small short arm. However, when describing ratios the letters S. A. (=short arm) have been used.

Satellite. The satellite extends from the nucleolar constriction region to the extremity of the short arm. A capital »S» denotes a large satellite and a small »s» a small satellite.

Ratio. This term refers to the ratio of the long arm/short arm without the satellite region. It is abbreviated in the text to the letters L. A./S. A.

Homozygous (*or homomorphic*) chromosomes. These terms have been used in the text with reference to the nucleolar chromosomes only and apply to those cases where the two homologous chromosomes in a given cell are morphologically alike.

Heterozygous (*or heteromorphic*) chromosomes. Again, these terms have been used to denote the presence of morphologically dissimilar but homologous (nucleolar) chromosomes, with special regard to the differences in the length of the arms, or to the differences in the size of the satellites. It should be noted that these two terms have not been used in their strictly genetical sense.

Aberration(*s*). The arms of the nucleolar chromosomes (and their satellites) in some of the inbred lines have been found to vary in length. These variations have been found to be dependent on the size of the knobs located in special regions of these arms. Such variations in length (due to differences in the knob sizes) have often been described in the text as aberrations. It is realized that this term is perhaps not the best one in describing the phenomena, because, as yet, no absolute proof has been obtained to show that such pheno-

mena are really structural in nature. Some indirect evidences, however, have been found and have been described under the section »Discussion».

Material and methods

The inbred lines of rye (Stålråg) examined in the present work, came from the same stock as that used for the previous investigation, and whose origin has been described in the previous paper (l. c.).

The following lines have been studied in the present investigation: Nos. 47, 84, 76, 71, 63, 39, and 98; all of which were grown in the year 1955. In case of one line, however, (line 47) the number of kernels available was rather small and so kernels of the same line, from the following year (1956) had to be used. Hybrids (F_1) between some of the above lines have been examined whenever it was necessary.

The population material that has been analysed here was harvested in 1954. Such an old stock of seeds had to be used because the variety (Stålråg) from which the inbred lines were derived, are not grown any more for commercial purposes. The last year when they were grown for such a purpose was 1954. Because of their relative age some of the kernels did not germinate, and hence the population analysis made in the present paper, should not be regarded as coming from a truly randomly selected material.

As in the previous investigation pretreatment of roots with a definite concentration of oxyquinoline (0.03 M/L) was found useful (*cf.* TJIO and LEVAN 1950). The time of treatment was about six hours, at 16° C for the inbred material, and at 13° C for the population. The chromosomes were subsequently stained with the aceto-orcein squash method. All measurements were made from temporary mounts. The method of measurement has already been described in the previous paper (*l. c.*). For pachytene preparations aceto-carmine squash method was used following fixations made in Carnoy's fixative (3: 1 Absolute alcohol: glacial acetic acid).

II. OBSERVATIONS

1. Mitosis in inbred lines

In Table 1 is given the distribution frequency of the long arm/short arm (minus the satellite region) ratios of the seven inbred lines. The lines have been arranged in order of decreasing ratios, line 47, having

TABLE 1. *Distribution frequency of the L. A./S. A. ratios of the nucleolar chromosomes of the seven inbred lines investigated in the present paper.*

Line No. (1955)	Ratio	1.5	1.6	1.7	1.8	1.9	2.0	2.1	2.2	2.3	2.4	2.5	2.6	2.7	2.8	2.9	3.0	3.1	3.2	N	$\bar{X}$	Group
47									4	5	4	7	5	4	4	0	2	1		36	2.49	
84								2	2	4	8	10	3	3	4	3	0	1		40	2.47	A
76									8	2	8	7	7	0	0	0	1			33	2.38	
71								10	17	11	8	6	1							53	2.12	B
63						6	10	12	9	1	4	1	0	2	0	0	1			46	2.11	
39		2	6	6	11	4	11	2	0	1										43	1.88	C
98			3	8	10	5	5													31	1.85	
Total for the 7 lines		2	9	14	27	19	40	42	23	33	31	16	9	8	3	4	2			282	2.20	

TABLE 2. *The values of »t» obtained on comparing the L. A./S. A. ratios of the nucleolar chromosomes of the seven inbred lines described in Table 1.*

No. of Chrom.	(40)	(33)	(53)	(46)	(43)	(31)		
Inbred Line No.	84	76	71	63	39	98		
	0.388	2.300	8.762	7.700	12.750	14.523	47	(36)
		2.181	9.971	8.318	14.463	16.757	84	(40)
			7.028	6.000	12.122	14.162	76	(33)
				0.472	7.394	9.679	71	(53)
					6.135	6.865	63	(46)
						0.750	39	(43)

In this and the following tables (Table Nos. 4, 5, 6 and 7) values of »t» above 3.460 indicate highly significant differences ($P < 0.001$). Values of »t» between 3.460 and 2.660 indicate significant differences ($0.001 < P < P\ 0.01$) and values of »t» between 2.000 and 2.660 indicate probably significant differences ($0.02 < P < 0.05$). Values of »t» below 2.000 are considered to be non-significant.

the highest ratio ($\bar{X}=2.49$) and line 98 having the lowest ratio ($\bar{X}=1.85$). In between lie the ratios of the five other lines. These seven lines can be conveniently arranged in three groups. The first group A, contains line Nos. 47, 84 and 76. All these lines possess a high L. A./S. A. ratio ($\bar{X}=2.4$ or more), and show a highly significant difference when compared to ratios of lines belonging to the other two groups. They show, however, a non-significant difference when the lines are compared amongst themselves. In Table 2 is given a summary of the »t» values obtained on comparing the ratios of the different lines. In the inner margin are given the identification numbers of the different lines. In the outer (peripheral) margin are given the numbers (in brackets) representing the total number of chromosomes that have been examined for the corresponding lines.

The same method has been followed in showing the »t» values of the total chromosome lengths, the long arm lengths, the short arm (minus sat. region) lengths, and the satellite lengths between the different inbred lines; these values are given in Tables 4, 5, 6 and 7, respectively. It can be seen from Table 2, that the value of »t» obtained on compar-

TABLE 3. *Summary of the lengths of the different regions of the nucleolar chromosome in the different inbred lines, described in the text.*

Line No.	Ratio L. A./S. A.			Tot. chrom. length			Long arm length			Short arm length			Satellite length			Group
	$\bar{X}$	m	N	$\bar{X}$	m	N	$\bar{X}$	m	N	$\bar{X}$	m	N	$\bar{X}$	m	N	
47	2.49	0.039	36	33.61	0.833	36	20.17	0.508	36	8.64	0.249	36	5.81	0.148	36	
84	2.47	0.030	40	34.01	0.635	39	20.53	0.379	40	8.80	0.167	40	5.65	0.125	39	A
76	2.38	0.031	33	32.35	0.401	33	19.08	0.289	33	8.50	0.115	33	5.77	0.079	33	
71	2.12	0.017	53	31.43	0.377	53	18.58	0.225	53	8.78	0.116	53	5.09	0.078	53	B
63	2.11	0.032	46	31.91	0.549	46	18.39	0.360	46	9.20	0.158	46	5.41	0.102	46	
39	1.88	0.028	43	33.38	0.419	43	19.08	0.238	43	10.85	0.218	43	4.45	0.047	43	C
98	1.85	0.022	31	31.63	0.747	31	17.92	0.470	31	10.21	0.259	31	4.53	0.073	31	

In this and subsequent tables all measurements are given in millimetres at a magnification of ca 3,500×.

ing line 47 and 84, is 0.388, and the total number of chromosomes examined is, N=40+36=76. This value of »t» is non-significant. On comparing lines 76 and 84 the value obtained for »t» is, »t»=2.181, P lying between 0.02 and 0.05 ($0.02 < P < 0.05$). A similar comparison with lines from the other two groups, gives a much higher degree of significance. Thus the value of »t» obtained when comparing line 84 and line 71 (belonging to group B) is 9.971 and this figure is highly significant ($P < 0.001$).

Under the second group (B) come lines 71 and 63, and they represent

TABLE 4. *Values of »t» obtained on comparing the total chromosome lengths of the seven inbred lines. (From the data given in Table 3.)*

No. of Chrom.	(39)	(33)	(53)	(46)	(43)	(31)		
Inbred Line No.	84	76	71	63	39	98		
	0.383	0.283	2.392	1.701	0.297	0.177	47	(36)
		2.213	3.502	2.542	0.827	2.423	84	(39)
			1.677	0.641	0.060	0.849	76	(33)
				0.733	3.473	0.235	71	(53)
					2.129	0.306	63	(46)
						2.048	39	(43)

TABLE 5. *Values of »t» obtained on comparing the long arm lengths of the seven inbred lines. (From data given in Table 3.)*

No. of Chrom.	(40)	(33)	(53)	(46)	(43)	(31)		
Line No.	84	76	71	63	39	98		
	0.565	1.868	2.862	2.851	1.936	3.249	47	(36)
		3.038	4.370	4.080	3.223	4.315	84	(40)
			1.362	1.483	0.014	2.096	76	(33)
				0.436	1.544	1.296	71	(53)
					1.597	0.797	63	(46)
						2.205	39	(43)

an intermediate group between the two other groups A and C. This group has a L. A./S. A. ratio around the value 2.1. As can be seen in Table 2 the differences between these two lines are non-significant, though highly significant differences are obtained when they are compared with lines belonging to the other groups. The same characteristic is shown in lines belonging to the third group C. This group belongs to the other extreme and has lines whose ratios are well below 2.00.

TABLE 6. *Values of »t» obtained on comparing the short arm (minus the satellite region) lengths of the seven inbred lines. (From data given in Table 3.)*

No. of Chrom.	(40)	(33)	(53)	(46)	(43)	(31)		
Line No.	84	76	71	63	39	98		
	0.537	0.507	0.525	1.888	6.677	4.376	47	(36)
		1.478	0.084	1.722	7.451	4.578	84	(40)
			1.736	3.569	9.549	6.042	76	(33)
				2.107	8.340	5.025	71	(53)
					6.145	3.347	63	(46)
						1.885	39	(43)

TABLE 7. *Values of »t» obtained on comparing the satellite sizes of the seven inbred lines. (From data given in Table 3.)*

No. of Chrom.	(39)	(33)	(53)	(46)	(43)	(31)		
Line No.	84	76	71	63	39	98		
	0.784	0.196	4.317	2.183	8.723	7.721	47	(36)
		0.804	3.871	1.497	9.023	7.738	84	(39)
			6.198	2.791	14.337	11.491	76	(33)
				2.563	6.934	5.168	71	(53)
					8.563	7.048	63	(46)
						0.897	39	(43)

The two lines representing this group here are line No. 39 ($\bar{X}$=1.88) and line No. 98 ($\bar{X}$=1.85). The difference between these two lines are non-significant (t=0.750).

In Table 3 is given a more detailed analysis of the lengths of the different parts of the nucleolar chromosome as found in the seven inbred lines. It can be seen that except for a few exceptions there is the same general trend for the different lines to keep to the original groupings, proposed for the ratios in Table 1. Some of these exceptions can be shown to be due to special reasons. Thus in line 39 (Figs. 6—9), the short arm often appears to have an extra fragment, lying between the satellite and the short arm region proper. Accurate measurement of this fragment was not always possible, with the result that in many instances the short arm (and the total chromosome lenght as well) has been given a slightly higher value, than is actually the case.

A brief outline of the main characters of the different lines is given below.

Line No. 47 (Fig. 1). This line has the highest L. A./S. A. ratio ($\bar{X}$=2.49) of all the lines examined. It also possesses the largest satellite ($\bar{X}$=5.81).

Line No. 84 (Fig. 2). This line has the longest total chromosome length ($\bar{X}$=34.01). This is probably due to the fact that it also possesses the largest long arm ($\bar{X}$=20.53) length. It has also a rather high L. A./S. A. ratio.

Line No. 76 (Fig. 3). This line possesses the smallest L. A./S. A. ratio of the three lines belonging to group A. It also contains the smallest short arm as well as the smallest long arm of all the lines belonging to this group.

Line Nos. 71 and 63 (Figs. 4 and 5). These two lines belong to the second group (B) and have intermediate L. A./S. A. ratios ($\bar{X}$=2.12 and 2.11, respectively). These values approach nearest the mean value obtained from the fifty population plants ($\bar{X}$=2.14) as is shown in Table 9. They also closely resemble the mean value obtained by pooling the ratios of all the seven inbred lines together ($\bar{X}$=2.20). This of course may be a pure coincidence. These two lines could be regarded as being »average lines», from which the amount of deviations present in the other lines could be measured.

In line 63, a knob-shaped projection has often been seen in the short arm of the metaphase chromosome. When such a structure is present, the short arm appears larger, and the corresponding value for the

TABLE 8. *Measurements of the different regions of the obtained from ten F_1*

		Hybrid											
		1	2	3	4	5	6	7	8	9	10	11	12
Ratio $\frac{\text{L. A}}{\text{S. A.}}$	98	1.8	1.6	1.9	1.7	1.8	2.0	1.8	1.5	1.6	1.8	1.7	1.9
	84	2.1	2.2	2.5	2.4	2.2	2.1	2.4	2.4	2.3	2.6	2.0	2.3
Total Chrom. length	98	33	34	30	40	32	35	33	35	30	33	28	31
	84	34	35	33	40	34	37	31	32	32	30	30	32
Long arm length	98	18	18	17	22	18	20	18	18	16	18	15	17
	84	19	20	20	24	20	21	19	19	18	18	16	18
Short arm length (minus sat. region)	98	10	11	9	13	10	10	10	12	10	10	9	9
	84	9	9	8	10	9	10	8	8	8	7	8	8
Satellite length	98	5	5	4	5	4	5	5	5	4	5	4	5
	84	6	6	5	6	5	6	4	5	6	5	6	6

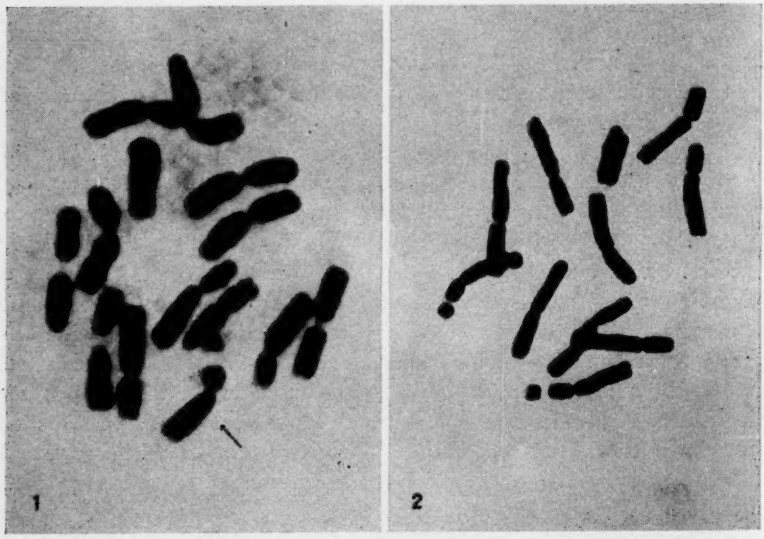


Fig. 1, somatic metaphase of an F_1 hybrid (76×47). The chromosome belonging to line No. 47 is below and is marked with an arrow (ca ×1600). — Fig. 2, somatic metaphase chromosomes of line No. 84 (ca ×1400).

two homologous nucleolar chromosomes in twentysix cells, hybrid plants (98×84).

cell No.																
13	14	15	16	17	18	19	20	21	22	23	24	25	26	$\bar{X}$	m	»t»
1.8	1.9	1.6	1.8	1.8	1.9	1.9	1.7	1.7	2.2	1.8	1.9	1.9	2.1	1.86	0.030	9.900
2.4	2.1	2.1	2.4	2.0	2.5	2.7	2.1	2.4	2.1	2.3	2.6	2.8	2.5	2.38	0.042	
38	27	25	30	32	33	30	34	35	37	36	36	43	29	33.62	0.801	0.583
40	30	26	29	32	33	31	36	36	37	39	34	44	33	34.35	0.782	
21	15	13	16	18	19	17	19	19	22	20	21	25	17	18.85	0.499	2.140
24	17	15	17	18	20	19	21	22	21	23	21	28	20	20.42	0.543	
12	8	8	9	10	10	9	11	11	10	11	11	13	8	10.65	0.270	4.625
10	8	7	7	9	8	7	10	9	10	10	8	10	8	9.08	0.209	
5	4	4	5	4	4	4	4	5	5	5	4	5	4	5.04	0.099	5.294
6	5	4	5	5	5	5	5	5	6	6	5	6	5	5.89	0.125	

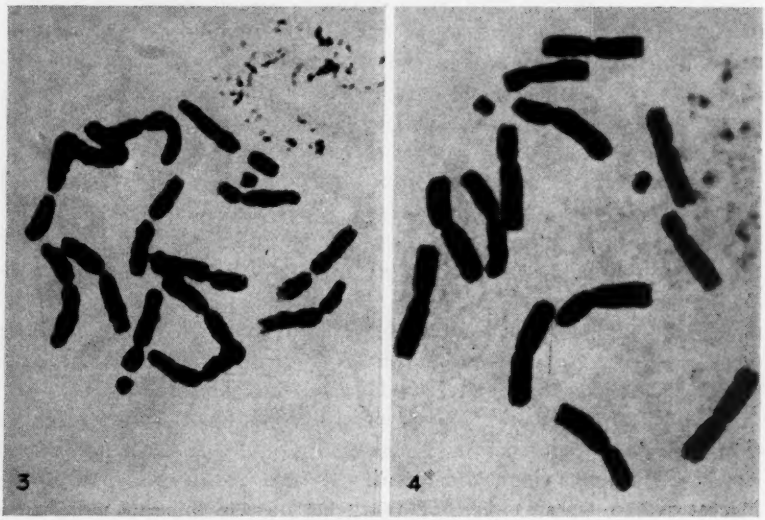


Fig. 3, somatic metaphase of line No. 76 (ca ×1700). — Fig. 4, somatic metaphase of line No. 71 (ca ×1700).

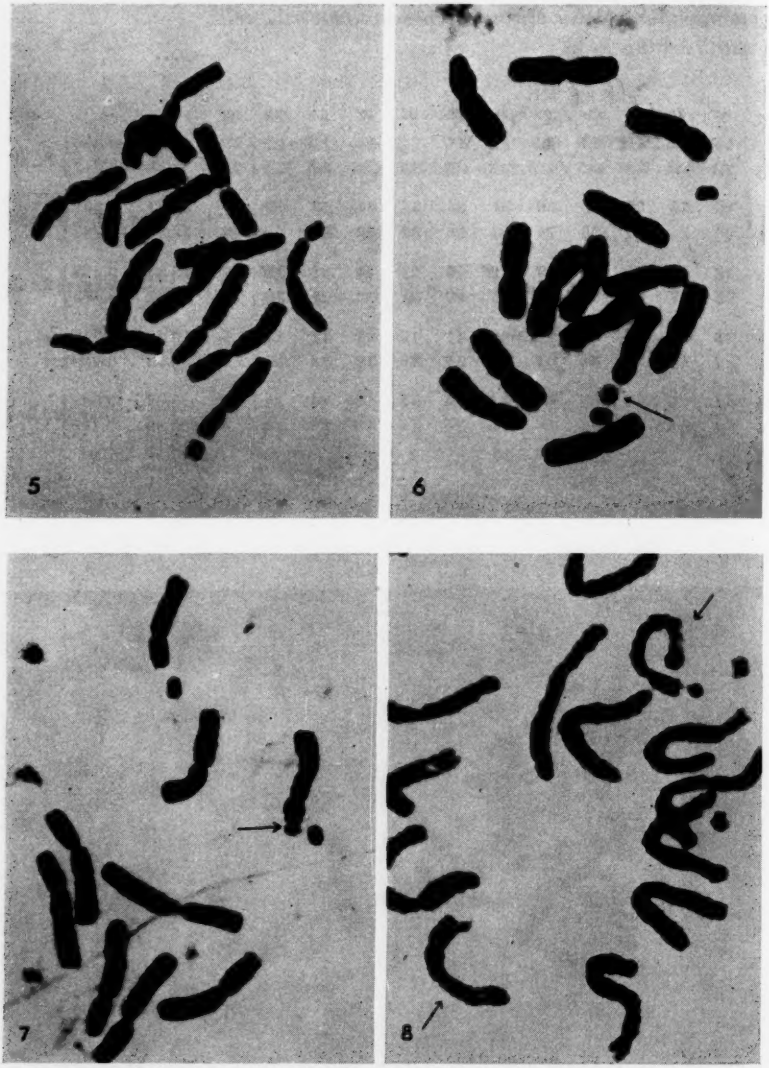


Fig. 5, somatic metaphase of line No. 63 (ca ×1700). — Fig. 6, somatic metaphase of line No. 39. Note the ball shaped fragment lying between the short arm and satellite in the nucleolar chromosome (marked by an arrow) (ca ×1700). — Fig. 7, somatic metaphase chromosomes of line No. 39. Note the knoblike projection (marked by an arrow) in the short arm of the nucleolar chromosome (ca ×1700). — Fig. 8, mitotic prophase of line No. 39. Note the fragments in the short arm regions of the nucleolar chromosomes. The nucleolar chromosomes are marked by arrows (ca ×1700).

L. A./S. A. ratio becomes smaller with the result that it often approaches the values obtained for the third group (i. e. less than two). Such a structure is usually present in one of the chromosomes only and may indicate the presence of some kind of heterozygosity in this line.

Line 39 (Figs. 6, 7, 8 and 9). This line is of importance, scince it throws some light on the structure of the short arm region. It has been mentioned before (l. c.) that the difference between a large short arm (»A») and a small short arm (»a») depended on the size of the knob present in the nucleolar organizing region. Observations made on the mitotic chromosomes of line 39, support this view. In Fig. 6 is shown a metaphase plate where one of the nucleolar chromosomes can be seen to have its short arm separated into two regions: (1) the short arm proper, lying next to the kinetochore, and (2) a small segment lying between this region and the satellite. In Fig. 6 this segment is seen as a separate spherical structure, and in Figs. 7, it is attached to the short arm. This structure can also be seen in the prophase of mitosis (Figs. 8 and 9). When ratios of arms are taken of chromosomes whose short arms show these segments, but excluding them in such measurements, they (i. e. the ratios) are found to approach the values obtained in lines belonging to group A (*cf.* Figs. 6 and 7). These knob-shaped structures and special segments have not yet been found in lines belonging to group A, though they have sometimes been found in line 63, belonging to group B and line 98 belonging to group C. In the author's opinion, the presence of these structures could be regarded as evidence of the presence of extra regions in the short arm. It probably indicates the presence of a kind of duplication in the knob of the nucleolar organizing region. It has also already been shown before (l. c.) that pachytene chromosomes of lines having large short arms show extra large sized knobs, in the nucleolar organizing regions (*cf.* Figs. 13, 14 and 15 in BOSE, 1956). These segments could then be regarded as parts of extra large sized knobs present in the pachytene stages of large short arm types of chromosomes.

In other respects this line has a relatively large short arm, ($\bar{X}=10.85$) a small satellite ($\bar{X}=4.45$) and a low ratio ($\bar{X}=1.88$).

Line 98. This line is characterized by its rather small long arm ($\bar{X}=17.92$), large short arm ($\bar{X}=10.21$) and small satellite ($\bar{X}=4.53$).

In Table 3, it can be seen that line 98 differs from line 84 in several respects. These are: (1) in their total chromosome length ($\bar{X}=31.63$ and 34.01 respectively), (2) in their long arm length ($\bar{X}=17.92$ and 20.53 respectively), (3) in their short arm length ($\bar{X}=10.21$ and 8.80 respec-

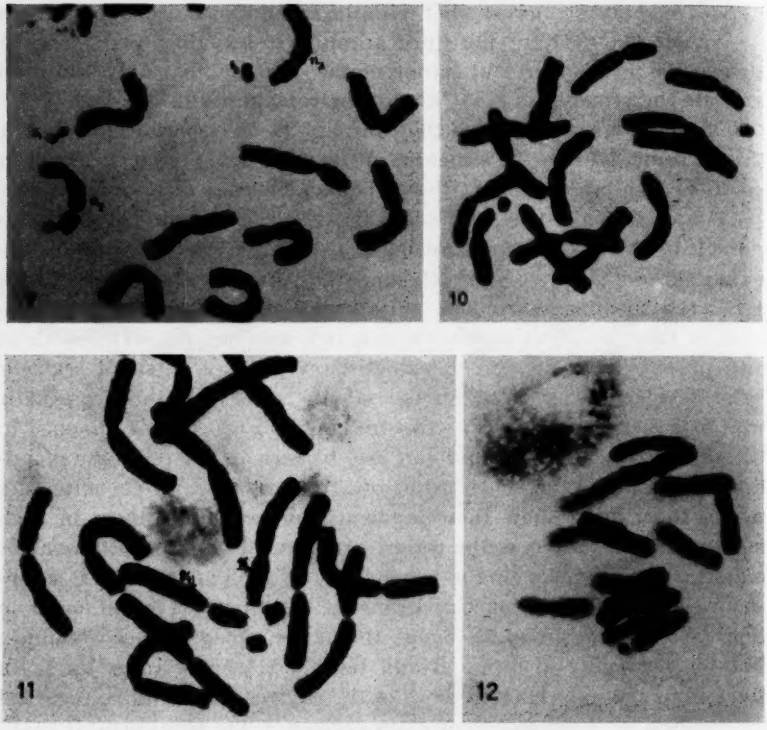


Fig. 9, mitotic prophase of line No. 39. The nucleolar chromosome to the left (n_1) has a small fragment attached to its short arm. Its satellite s_1 can be seen lying above. The short arm of the other nucleolar chromosome to the right (n_2) has a knoblike structure which has not yet separated. s_2 is its satellite (ca ×1400). — Fig. 10, somatic metaphase of line No. 98 (ca ×1500). — Fig. 11, somatic metaphase chromosomes of an F_1 hybrid (98×84). The nucleolar chromosomes belonging to the two lines are indicated by their respective numbers. Note the differences in the long arm lengths, short arm lengths and satellite sizes. The smaller satellite belong to line No. 98. Note also the knoblike projection in the short arm of the chromosome belonging to line No. 98 (ca ×2200). — Fig. 12, somatic metaphase of a population plant. Note the differences in length of the short arms and the satellites in the two nucleolar chromosomes (ca ×1200).

tively), (4) in the size of their satellites ($\bar{X}$=4.53 and 5.65 respectively), and finally (5) in the ratios of their arms ($\bar{X}$=1.85 and 2.47 respectively). From Tables 4, 5, 6 and 7, it can be seen also that all these differences are rather highly significant in nature except perhaps the differences found for the total chromosome lengths. The estimated

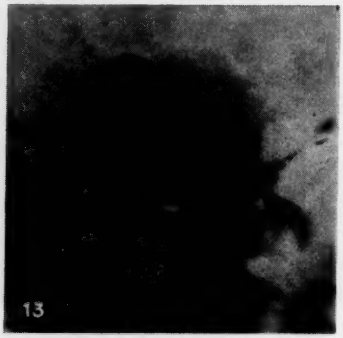
value for »t» in this case is 2.423 ($P < 0.05$). The estimated value for »t» for the observed differences between the long arm lengths is 4.315 ($P < 0.001$).

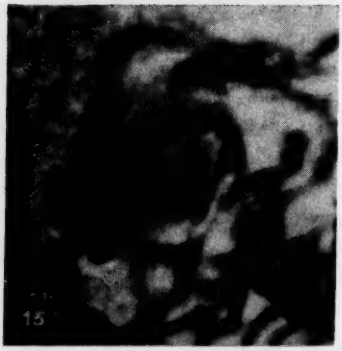
In order to find out whether the total length of the chromosome as well as the length of the long arm, were really different in the two lines, some of their F_1 hybrids were examined. It was possible to use as markers such easily identifiable characters as the L. A./S. A. ratio, short arm size and satellite size, and, in this way to separate the chromosomes belonging to the two lines, in all the complete cells of the hybrids that were examined. In Table 8 is given the results of such an examination. Here measurements of the different regions of the chromosomes, from twenty six complete cells are given from about ten hybrid plants.

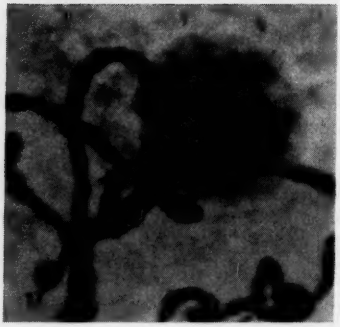
It can be seen that clear differences exist in every cell with regard to the following characters (1) Ratio of L. A./S. A., (2) Long arm length, (3) Short arm length, (4) Satellite size. Though the mean value of the total chromosome length of line 84 showed a slightly higher value than that of line 98 ($\bar{X}=34.35$ and 33.62 respectively), this difference is not significant. It is possible, however, that if a larger number of measurements were made, there would have been a significant difference. Fig. 11 is a cell from such an F_1 hybrid plant, where all the differences in the various regions of the nucleolar chromosomes that were mentioned above are clearly seen.

2. Pachytene studies

In the paper published before, mention was made of the size differences that had been found in the knobs of the pachytene chromosomes of some hybrids of inbred lines and also in some of the plants from the population. The data collected at that time were too meagre to allow any definite statement to be made. Since then, however, more pachytene observations have been made on plants having different chromosome types, and the results of these observations are described below. Figs. 13 and 14 show the nucleolar chromosomes in the pachytene stage, of a population plant, where the terminal knobs and the knobs present in the nucleolar organizing region are seen to be of nearly the same size. The mitotic configuration of the nucleolar chromosome of this plant is not known but is probably of a type belonging to group (B) or group (A). In the pachytene configuration shown in Figs. 15 and 16, the terminal knob of the nucleolar chromosome can also be seen to be nearly equal in size to the knob found in the nucleolar organiz-







Figs. 13—18. Microphotographs and respective drawings of the nucleolar chromosome in the pachytene stages. Figs. 13—14, population plant. — Figs. 15—16, F_2 hybrid having an aS/aS type of chromosome configuration. — Figs. 17—18, inbred line No. 121 (1951) having an As/As type of chromosome configuration (ca ×2900).

ing region. The mitotic chromosomes of this plant have been studied and found to have small short arms and large satellites. This plant is an F_2 hybrid derived from a cross between two inbred lines (78×121——1952) of which line 78 had small short arms and large satellites (aS/aS) and line 121 had large short arms and small satellites (As/As). This plant therefore resembled one of the original parental types, i. e. line 78 (aS/aS). The manner in which such F_2 plants were obtained has been fully described before (l. c.). Finally in Figs. 17 and 18 are shown the chromosomes of a plant belonging to inbred line No. 121 (1951). This plant has a chromosome configuration of As/As type, (i. e. large short arms and small satellites). As can be observed from Fig. 14, a clear difference is present in the size of the knob present in the nucleolar organizing region and the knob located in the satellite region. The knob in the nucleolar organizing region is much larger in size than the satellite knob. Thus, we can confirm the conclusion arrived at before that the size of the knob present in the short arm, is responsible for the size of the arm. It is not possible to show the connections between the size of the satellite knob and the size of the satellite, in pachytene, but it has been shown before (l.c.) that a large knob is present in a large satellite which can be clearly seen in the prophase stages of the mitosis. This knob is extremely weak, however, in a plant with a small satellite.

Observations made on the lines studied in this paper have also confirmed the above facts i. e. that lines having large satellites have large knobs and vice versa.

It is more difficult, however, to account for the differences in the long arm lengths. In one case, however, in a hybrid with one homologue having a large long arm, and the other homologue having a short long arm, it could be seen that the larger long arm, had also a larger knob, located in the terminal position of the arm.

Though no pachytene analysis has yet been made in line Nos. 39 and 98, the frequent presence of knoblike structures and even fragments in their short arms, probably indicate the presence of large knobs in the nucleolar organizing region and which are probably similar in nature to those of line 121 (1951).

3. Population data

In order to explain the presence of some of the aberrant types of chromosomes in the inbred lines described before, it was thought necessary to examine samples of population plants. A preliminary re-

TABLE 9. ***Showing the distribution of the nucleolar chromosomes, according to their L. A./S. A. ratios, of fifty population plants.***

(The numbers in the table refer to the serial numbers of the fifty population plants examined. Every number is repeated twice, and each represents a homologous chromosome. For further explanations see text.)

L. A./S. A. Ratio:	1.4	1.5	1.6	1.7	1.8	1.9	2.0	2.1	2.2	2.3	2.4	2.5	2.6	2.7	2.8	2.9	3.0	3.1	
						4	3	2	2	1	4	1							
				9		9	3	26	10	18	10	13	13				26		
					36	24	5	36	5	18	11	22		24					
					44	44	6	6	7	47	11	22							
					31	20	20	16	7	47	16	31							
							23	23	8	19	19		8						
						27	30	27	12	30	12								
						46	32	14	14	25	25								
						46	32	28	15			28	15						
					37		33	37	17	17	39	33	39						
					21		43	43	21										
			48					48	29		29								
							50	50	34		34								
							35		35		45								
								38	38		45								
							49	40	40		49								
							41		41										
									42	42									
			1	1	5	8	15	15	18	10	14	7	4	1	0	0	1	$\bar{X}=2.14$	

TABLE 10. *Different types of plants found, and the frequency in which they occurred, in a sample of fifty popoulation plants.*

	No. of plants	Group
I. Plants with homozygous (or homomorphic) nucleolar chromosomes		
1) Plants having L. A./ S. A. less than 2; with large short arms and small satellites (As/As)	5	C
2) Plants having L. A./S. A. more than 2.3; with small short arms and large satellites (aS/aS)	10	A
3) Plants having L. A./S. A. intermediate to (1) and (11) and equal to ca 2.1; short arms and satellites intermediate	4	B
II. Plants with heterozygous (or heteromorphic) nucleolar chromosomes		
4) Plants having short arms and satellites both heterozygous	16	—
5) Plants having satellites heterozygous only	6	—
6) Plants having short arms heterozygous only	2	—
III. Plants uncertain as to which group they should belong	7	—
	Total 50	

port was given in the previous publication, and a more detailed analysis has been given below.

Data from fifty population plants have been presented in Table 9. Each population plant has been given a number running from one to fifty. Every number has been represented twice so that they represent the two homologous chromosomes. Each chromosome (i. e. number) has been placed under the particular ratio it has been found to have. To take an example, plant No. 1 (one) has one homologue having a ratio between 2.2 and 2.3 and the other homologue having a ratio between 2.4 and 2.5. Hence in Table 9 the two »ones» have been so placed, that each is under its respective ratio.

Plant No. 1 has been considered to have some degree of heterozygosity. This is not only because their homologues differ in their ratios (this is due to differences in length of their short arms) but also because they show differences in the size of their satellites.

In Table 10 is shown the different types of homozygous and heterozygous plants that have actually been found amongst the fifty population plants, together with the frequency in which they occurred. To simplify matters, measurements of the long arms have been neglected in the classification given in this table, the main criteria used being the L. A./S. A. ratios, the size of the short arms and the size of the satellites.

The last line of Table 9 gives the general distribution of the ratios

of the one hundred chromosomes, examined from the fifty population plants. It may be pointed out, that this distribution curve and the mean ratio ($\bar{X}=2.14$) closely resembles the distribution curve and mean obtained by adding the ratios of the seven inbred lines together ($\bar{X}=2.20$, Table 1, last line).

As will be seen from Table 10 about one half (24/50) of all population plants have heterozygous chromosomes. On selfing such plants progenies having homozygous chromosomes can be expected to occur. In this way the origin of different types of inbred lines may be explained. The existence of a mechanism (crossing over in the nucleolar organizing region) by which such types of plants may occur has been demonstrated in the previous paper (BOSE, l. c.).

The number of plants having small short arms and large satellites are the most common amongst the homozygous types obtained (10/50). Whether this indicates that they may have a better survival value is not known.

III. DISCUSSION

From the data presented in the previous section, the following conclusions can be drawn:

The differences in the lengths of the short arms and satellites, that have been shown to be present in some of the inbred lines, are dependent on the size of the heterochromatic knobs present in special regions of their arms and satellites. The knobs of the short arms are located in the nucleolar orgainzing region, and the knobs of the satellites are terminal in position. Due to the tendency of the heterochromatic regions to contract less than the surrounding euchromatic regions arms containing large heterochromatic knobs appear relatively larger in the highly contracted metaphase stage than in the earlier and more distended prophase. This may partially explain also why F_1 hybrids of lines having different arm lengths or satellite sizes do not show very much abnormal configurations in the pachytene. However, this difference in the size of arms as seen in the metaphase stages between two heteromorphic chromosomes (of an F_1 plant) should be regarded as being both due to the difference in the total amount of knob material as well as due to differential contraction between the euchromatin and heterochromatin regions.

Two hypotheses may be put forward to explain the origin of the observed differences in the knob sizes. The first one is that these size

differences are genetically controlled, that is they are controlled by genes located in the same region as the knobs. In the simplest case a single pair of genes may be regarded as being responsible for the character largeness or smallness of knob size. Since both types of chromosomes (i. e. one chromosome with a large knob and the other with a small knob) can occur in the same cell, such genes must be regarded as having extremely localized effects and also as showing no dominant or recessive expression.

There seems to be a quantitative gradation in the size of the arms (and presumably in the knob size) within the different lines, and to explain this a kind of multiple gene hypothesis may have to be assumed. Since frequent crossing over involving these knobs occur, these genes must be regarded as being located rather near the knobs they are controlling.

The second hypothesis is simpler and more acceptable. Here the variation in the knob size is regarded as simply being due to structural changes. It had been shown before (LIMA-DE-FARIA, 1952) that the knobs of rye are composed of an association of several chromomeres. If then the differences in the size of the knobs could be shown to be due to the number of chromomeres present within these knobs, such differences could be regarded as structural and should be called duplications or deletions. As yet no definite evidence of the variation in the chromomere numbers in the knobs of different sizes have been found. This has been mainly due to difficulty in getting good pachytene preparations in many of the lines. It is hoped, however, that a suitable technique will be evolved to overcome this difficulty. There are, however, some indirect evidences to support the second hypothesis.

It will be recalled that in the short arms of such inbred material as line 39, 98 and even line 63, small knob-shaped projections can sometimes be seen, in the metaphase and prophase stages of mitosis. In extreme cases they can be seen as small fragment-like segments lying between the short arms and satellites. These segments are partially due to fixation and subsequent pressure from squashing, but there can be no doubt that they indicate, that in the short arms of these lines, there exist complex structures not found in lines having small short arms. Since similar structural differentiation has not been found in lines with small short arms, one cannot avoid concluding that these differences in size between small short arms and large short arms have been caused by the addition of extra material in these regions. This could then be regarded as a kind of duplication.

LONGLEY (1937) showed that bending and twisting occurred in pachytene chromosomes (of teosinte) where one of the homologues had a knob present in a particular region, and which was absent in the same region, in the other homologue. Similar bends and twists have been observed in rye hybrids, having As/aS and AS/as types of chromosomes (*cf.* Figs. 6—11, BOSE 1956). The phenomenon of differential contraction between the heterochromatic and euchromatic regions of the chromosomes, can also partially account for the fact that no typical deletion loops occur in the knob formation regions during the pachytene stage.

WHITE (1954) has given an excellent review of the occurrence of heteromorphic chromosomes in animals and especially in insects. Here too the unequal bivalents, found in meiosis, have been shown to be due to the differences in the size of heterochromatic regions. He suggests their origin mainly through duplication and has also considered the possibility of these regions arising through translocation from B chromosomes. It is interesting to note that many of the insect species having unequal bivalents have »B» chromosomes. It is well known, of course, that B chromosomes are also found in rye.

SUMMARY

(1) In this paper a detailed account of measurements made on the nucleolar chromosomes of seven inbred lines have been presented. It has been shown that a considerable variation exists in the L. A./S. A. ratios of the nucleolar chromosome in the different inbred lines.

(2) It has been possible to divide these seven lines into three groups, on the basis of the value of their L. A./S. A. ratios. The first group (lines 47, 84 and 76) contain those lines whose ratios are greater than 2.4. The second group contains those lines (Nos. 39 and 98) whose L. A./S. A. ratios are less than 2.0, and the third group includes lines whose L. A./S. A. ratios lie in between the first two groups (i. e. 2.1). The two lines that were examined in this group are Nos. 71 and 63.

(3) These variations in the ratios were found to be caused by variations in the sizes of the arms (mostly the short arms) in the different lines. Variation in size has also been found in the satellites. The cause of such variation in length was due to differences in the size of knobs, present in definite regions of these arms and satellites.

(4) These variations in size of the arms and satellites, or in the size of the knobs of the arms and satellites have been considered to be struc-

tural in nature and hence the term »aberration» has been used. Their nature and origin have been discussed.

(5) An analysis of fifty population plants has revealed the presence of similar types of aberrations in either one or both of the homologous chromosomes. About one-half of these plants were heterozygous for such aberrations.

(6) It is considered that many of the aberrations found in the inbred lines, were derived from the population, where they were already present. It is suggested that such aberrations may have played some role in inbreeding effects.

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MUTATIONS INDUCED BY THE ACTION OF METAL IONS IN PISUM

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I. GROWTH STIMULATION

MOST of the elements of the periodic system with an atomic number less than 30 frequently occur in the earth crust. Several of these elements, H, C, N, O, P, S, belonging to the group of metalloids at the same time form important bases for all living matter (»nutrients»). Other elements as K, Na, Mg, Ca, are important constituents in the living cell system, especially in the cell walls. This group of light metals is usually called »macro-elements», because their content in the living organism is rather high, in contradiction to the following group. The last group, called the »micro-element group», comprises those elements of which a living cell system needs only very low concentrations for a normal metabolism. This group comprises among others the following typical metals, namely Cu, Zn, Mn, Fe, Co and Ni. The members Mg and Ca of the former group are known to form weak chelates or complexes similar to chelates with proteins in the cell. All the metal ions of the third group form over $P_H 5$ typical chelate compounds with a high or rather high stability constant. It is moreover known that several typical enzymes in plants and animals are constructed through the principle of chelates. Examples of those metals which form an integral part of a chelate compound are Fe, Mg and Co in porphyrins; Mg, Mn and Zn fixed to proteins as enzyme activators and Cu in oxidases (BURSTRÖM and TULLIN, 1957). In the living cell the total content of each of these metals must be balanced in an effective, reversible and at the same time sensitive system. Surplus or deficit in a plant of an important element (or its chelate component) would produce poison symptoms and decrease the vitality. This is a well known phenomenon both theoretically and practically. Some of the necessary bivalent metal ions, especially Ca and Mg, but also to some degree Zn and Mn, (Al, too, which normally is poisonous to the plant), produce on *Pisum* plants and

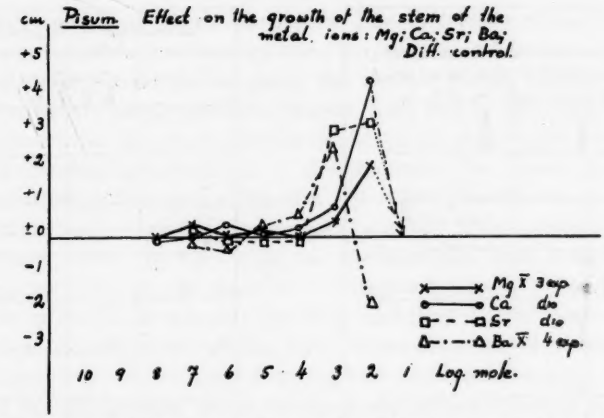

Fig. 1. The net growth increase of the stem length by treatment with alkali earth metals. *Pisum sativum.*

some other tested species a marked *increase of the length growth of the stem* (VON ROSEN, unpubl. data). It may be mentioned that on rootlets of wheat seedlings, Ca, especially, increases the cell elongation (BURSTRÖM and TULLIN, 1957).

A few of these experiments may be briefly reported here to demonstrate the importance in the cell tissue of some of those elements which have given significant effects. The method used in the experiments was treatment of germinating seeds of different species, above

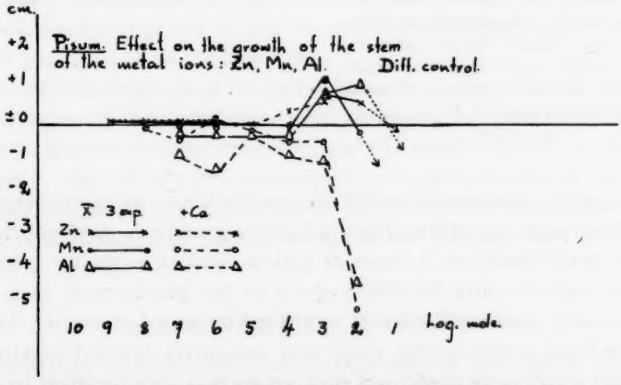

Fig. 2. The net growth increase of the stem length by treatment with some metals: Zn, Mn, and Al. *Pisum sativum.*

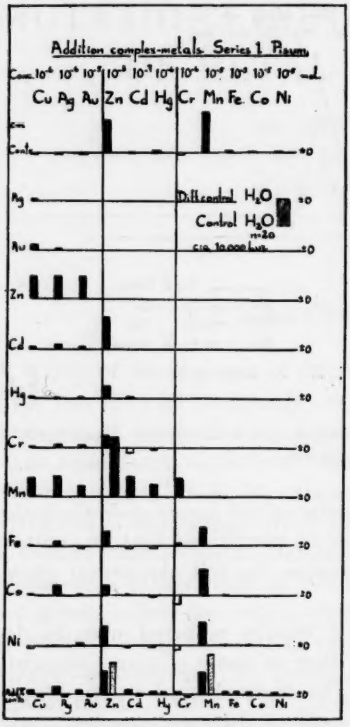
Addition complex-metals Series 1 Pisum
Cu Ag Au Zn Cd Hg Cr Mn Fe Co Ni
Diffcontrol H_2O
Control H_2O
Cu Ag Au Zn Cd Hg Cr Mn Fe Co Ni

Fig. 3. Schematic schedule of an addition series of typical complex-forming metals. Net growth increase of the stem length. *Pisum sativum*. Black columns upwards demonstrate increased growth, white columns downwards decrease of the combination effect.

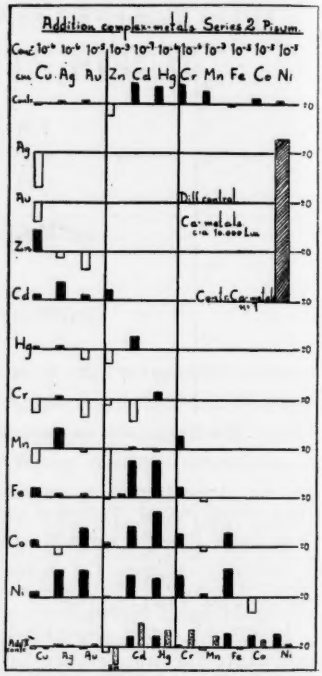

Fig. 4. The same schedule of addition as in Fig. 3, but in the presence of alkali earth metals in the conc. 10^{-3} mole/lit.

all peas, with a decreasing series of concentrations of metal solutions in the form of salts of nitrate. During further growth in daylight the seeds grew in petri dishes on a layer of cotton, soaked with the same metal solution, and the only fertilizers given to the plants were members of the necessary nutrients without any kind of metal elements. After ten days the total length of the plant was measured. Several repetitions of every test series were made and each series was accompanied by control dishes, only treated with dist. water and the base nutrient solution. Each

petri dish contained about 50 plants. The difference found in plant length was calculated on the net stem length, *i. e.* the length of the test plants subtracted from the length of the control plants. About 50 of the normal elements in the periodic system were tested with this method. The elements not discussed here have shown no statistically significant effect increasing growth. Fig. 1 demonstrates the great growth-increasing effect of the group Mg—Ca—Sr—Ba, which in the periodical system are situated together in the same column. The concentrations at the optimal effect are very high for the elements with a low atomic number, but decrease for those with a higher number. These elements are weak or not at all complex-forming, and they are not radiomimetic.

The next Figure, 2, shows the same effect for some elements, which at the same time are complex-forming and thus radiomimetic, *viz.*, Zn, Mn and Al. The growth effect for these metals is somewhat less pronounced. When calcium in the concentration 10^{-3} mole/lit is present at treatment, the growth-increasing effect is nearly totally lost. (This observation seems to be a special problem, which will not be discussed here.)

Several experiments with the addition of two metals or combination of up to ten metals simultaneously were made. Figs. 3 and 4 from the addition treatments may demonstrate at first that several metal ions do show growth-increasing effect when combined with another metal, even when they were inactive in single treatments, *e. g.*, Au, Cd, Cr, Co. (In the figures the black columns upwards demonstrate the length in cm. of increased growth in comparison to the controls, and the white columns downwards demonstrate combinations, where the growth is worse than the control.) In Fig. 4 calcium is added to the solution. The reactions of several of the metals are quite different in comparison with those of Fig. 3, *e. g.*, Zn! Mn! Fe, Ni.

These demonstrations of increased growth of the plant by treatment with some metal ions and thus simultaneously of actions in the cells may be important for our knowledge of the whole system of metabolic processes in the cell. Future scientists may be able to explain the theory of these reactions. They are certainly of interest for understanding the activity of the complex-forming metals in the cell and their presence in enzymes of chelate type.

II. RADIOMIMETIC REACTIVITY

On seedlings of *Pisum* and some other species the author has tested about 50 of the normal elements in the periodic system as to their radiomimetic reactivity (VON ROSEN, 1954 a and b).

The results of these experiments have indicated that all those metal elements which chemically are able to form strong complexes with proteins (aminoacids, aminobasis, cyclic compounds and others) are also radiomimetically reactive. Examples of those metals are Cu—Ag—Au: Zn—Cd—Hg; Ga—In—Tl; Cr, Mn, Fe—Co—Ni; Pd and Pt. Note the combination in chemical groups. They were if possible tested as nitrate salts. The observed cell disturbances are of the same kinds as are known from treatments with ionizing radiation or chemical agencies such as *e. g.*, mustard gas. Weak *e. g.*, Mg—Ca—Sr—Ba, or non-complex-forming elements (*e. g.*, Na—K—Rb—Cs) produce very few or no chromosome breaks at all. The radiomimetic reactivity is probably not specific to any one element, but not additative. The optimum frequency of disturbed cells with fragments, bridges and other abnormalities is much lower than usually occurs as a result of ionizing radiation. A poison effect which breaks the cell metabolism down to pycnosis is certainly the reason for the low frequency of visible chromosome breaks. In all mutation experiments it is a fundamental experience that the appearance of artificially induced gene mutations is normally accompanied by chromosome disturbances or vice versa. Such is the typical consequence of all ionizing radiation or treatments with mustard gas and ethylene oxide, but *not* with 8-ethoxycaffeine and *nebularine* (see EHRENBERG, GUSTAFSSON and v. WETTSTEIN, 1956). The investigations of the genuine mutagenic effect of the radiomimetic metal ions have therefore a considerable interest, not only from the theoretical, but also from the practical point of view.

III. MUTAGENIC EXPERIMENTS

For special reasons the variety *Pisum abyssinicum*, BRAUN was used in all the mutagenic experiments. It is a very favourable plant for this type of investigation. Furthermore, this particular species was the main material for the radiomimetic investigations (VON ROSEN, 1954 b). Its genetical and cytological constitution in comparison to normal *Pisum sativum* and other *Pisum* species is well established (VON ROSEN, 1944). The first experiments on induced mutations were carried out by steeping germinating seeds in a combination of nitrate salts of different reactive metal elements and propagating the living plants from these seedlings. No morphological mutations at all were, however, to be seen when 84 plant progenies were checked in X_1 and X_2. The second experiment series

included repeated spraying of a mixed solution of 10 different reactive metals as nitrate salts on two to four-week-old plants. The X_1 sterility was insignificant. 100 family progenies from treated X_0-plants were observed in X_2 and X_3, from which 3.0 % segregated chlorophyll defective individuals. Among these pure *albina* appeared. That type is extremely rare in the genus *Pisum*. In the third experiment young buds in the bud cluster were, during the time of their meiosis, given repeated injections of the same metal solution as used in the above-mentioned experiment. The X_1 sterility was low. Of the 83 observed plant progenies 3.6 % had mutated.

The next step was to find a method which would allow the metal ions to enter the plants more easily and to affect the generative cells during praemeiosis and meiosis; that is to treat the root with the metals, instead of the wax-covered leaf surface, and simultaneously to treat the plant so effectively that it would be semivital and thus more sensitive. A method which in principle corresponds to these requirements was after several preliminary experiments found when treating with a metal ion solution the contents of clay jars in which 2—3-week-old plants were growing. The pots were filled either with normal soil or with vermiculite. The concentration and doses of the metal solution depended upon the vitality reactions of the plants. None or very little nutrient fertilizer was given to the plants. It was not difficult to follow the effects of the metal ions on plant growth and vitality.

This experiment was divided into three parts: A) One part was kept as control. From 56 plant progenies no visible real mutation appeared in X_2 or X_3 (X_3 was grown 1957). B) The second part of the clay jars was treated with a solution of salts of nitrate of combined light and heavy metals containing typical radiomimetic metals as Cu, Zn, Cr, Mn, Fe, Co, Ni, Al and Li with a low atomic number and Ag, Cd, Hg, Tl with a high atomic number. The effect on the X_0 individuals (=the treated plants) ranged from an obviously decreased vitality to lethality, dwarf characers in growth development, marked decreased size of the flowers and other phenotypic reactions. The sterility was high in the microspores as in the seed setting. In X_1 and X_2 the plants showed a weak vitality but a regular growth. The seed setting was not quite normal either. Of 64 plant progenies followed to X_3, 4.7 % had mutated. C) The third part of the series was treated with only those metals which are characterized both as necessary »micro-metal-elements» from a physiological point of view and as typical radiomimetic elements. The combination used contained the following metals: Mg, Ca, Cu, Zn, Mn, Fe, Co and Ni. The

concentrations used corresponded to those given in B). The plants showed a significantly increased vitality, higher and more luxuriant habitus than the control jars and excellent seed setting. In the next two generations this luxuriant growth was not so prominent but anyhow clearly visible. Also this year, in X_4, the plants of group C) showed a slightly increased vitality in comparison with the other two groups. Of the controlled 176 plant progenies 8.5 % had mutated.

All the mutations which appeared in group B) were chlorohpyll mutations. In group C) the main type was of the same kind (=6.8 % mutated treated plants). The rest of the mutants contained a waxless group which more or less lost its coat of wax on the leaflets. This morphological type is a wellknown spontaneous mutation in *Pisum sativum* which normally segregates monofactorially. There were no differences in mutation rate between the plants which had been grown in normal soil or in vermiculite. VON ROSEN, 1954 b, and in press has described some »Gigas» and »Dwarf» mutations, originating from treatment with P^{32} and mustard gas. It is fairly possible that similar »Gigas» mutations have appeared after treatment with metal ions. The proof of these observations will, after repeated pedigree selections, come in the following generations. *Thus by the method used gene mutations have been induced in peas with single metal ions, representing normal elements necessary for every living organism.* The metal compounds, used up to now, have been the salts of nitrate. Experiments using Me-EDTA (=ethyldiaminetetraacetic acid) are now being carried on, while the Me-EDTA compound probably enters the plant more easily than the (Me^{++}).

It has been possible to compare the morphological types of mutation, appearing after treatment with metal ions, with those induced by some other common agencies. Formerly the author has carried out irradiations on *Pisum abyssinicum* with X-rays (on a small scale), β+particles and treatment with mustard gas and mustard oil (VON ROSEN, 1954 b) and also in recent years with fast neutrons (unpublished data). The comparison may also be completed with the results from X-irradiation on *Pisum sativum* and of the combination of data from the literature about observed spontaneous chlorophyll mutations in peas (VON ROSEN, 1942).

Table 1 shows the qualitative rate of different induced mutations. It is clearly demonstrated in the Table that all those kinds of mutation, especially chlorophyll individuals, which appear spontaneously or after ionizing radiation, are also observed after treatment with metal ions.

TABLE 1. *Qualitative rate of different induced mutations.*

	Albina	Xantha	Viridis	Virescens	Semi-vit.	Wax-less	Gigas	Dwarfs	d → D	Semi-ster.	Radiom. effect
Pisum sativum											
Spontaneous	+	+	+	+	+	(+)	(+)	+	?	(+)	−
X-rays	−	+	+	+	+	−	?	?	?	+	+
Pisum abyssin.											
Control	−	−	−	−	−	−	−	−	−	−	−
X-rays	+	−?	+	+	+	?	+	−	−	+	+
Fast neutrons	−	+	+	+	+	+	?	?	?	+	+
β + − particles (P^{32})	−	−	+	+	+	−	+	+	+	+	+
Mustard gas	−	−	+	+	+	−	+	+	+	+	+
Metal ions [+]	+	+	+	+	+	+	?	?	−?	−	+

TABLE 2. *Mutation rate (%) of different induced chlorophyll mutations.*

	Albina	Xantha	Viridis	Virescens	Maculata	Total No. of mutat.	No. of plant progenies examined.
Pisum sativum							
Spontaneous	+	+	+	+	+	139	?
X-rays	—	0.2	0.4	0.8	0.1	21	1348
Pisum abyssin.							
X-rays	0.6	—	1.2	—	—	3	160
Fast neutrons	0.4	1.2	3.6	0.4	0.4	15	250
$\beta+-$ particles	—	—	21	4	—	51	193
Mustard gas	—	—	31	10	—	44	106
Metal ions^{++} I	1.1	0.5	0.5	1.1	—	6	183
II	—	1.2	2.5	2.5	—	15	240

The results from *e. g.*, the comprehensive mutation investigation on barley of GUSTAFSSON and his collaborators in Sweden demonstrate similarly the same qualitative representation of morphological types induced by different kinds of treatment. The mutation rate frequency in per cent of the different induced types of chlorophyll mutations is demonstrated in Table 2. The comparison between different agencies is comparable to that of Table 1. Table 2 comprises, however, only the chlorophyll mutations. Of course it is impossible to give any data about the number of plant progenies behind the observations of the spontaneous mutations. In the group »metal ions^{++}» sign I means experiment series two and three mentioned above with spraying of metals on young growing plants and includes these two series together. Sign II contains the two soil treatment series. The Table demonstrates that the metal ions induce the same principal frequencies of the different types of mutations as do the other compared agencies. The frequency of *chlorina* mutations (*i. e. viridis* and *virescens* types) among descendants from treatments with β-particles and mustard gas is indeed on a characteristic high level. A total break down of the green chlorophyll apparatus will not be realized and thus *albina* or *xantha* types never appear.

Fig. 5, at last, gives another representation of the comparison of the effect of different kinds of treatment. The spectrum of the different chlorophyll mutations, assigned in per cent of the total number of mutations found in each treated group, is given in the diagram. In the first place it is specially interesting to observe that the spontaneous and always very rare *albina* type appears in a high frequency in the metal ion

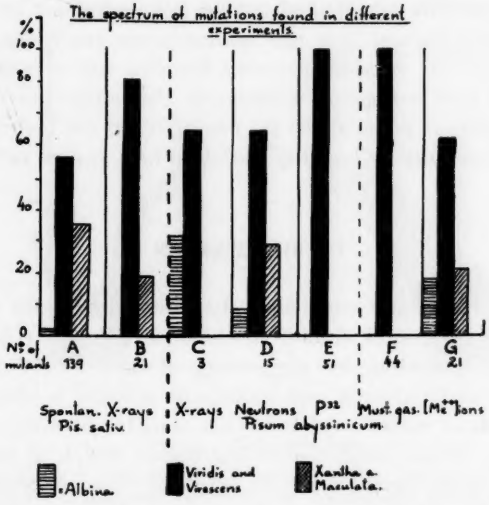

Fig. 5. The spectrum of mutations found in different experiments.

group G. The indications in group C (X-radiation on *P. abbyssinicum*) are insignificant owing to the very low number of observed chlorophyll-deficient individuals. The second observation comprises the fact that neither in group E nor in F (P^{32} and mustard gas resp.) have any white or yellow mutants appeared. EHRENBERG, GUSTAFSSON and v. WETTSTEIN, 1956 also report similar essential differences in their barley series. There it is the organic compound nebularine which clearly differs from the other agencies; but mustard gas also shows to some degree the same divergence. From these data it is obvious that the mutagenic effect of the metal ions is similar to the action of strong irradiation with X-rays or fast neutrons. Thus, the softer activity of β-particles and mustard gas gives another mutation spectrum. Possibly very sensitive and specific enzyme systems, which take part in the synthesis of the green chlorophyll apparatus, or the chlorophyll apparatus itself, are directly attacked by these soft agencies. It may be mentioned that all the ionizing radiations were performed on dry dormant seeds, while mustard gas and metal ions have been used on growing plants. Notwithstanding, the metal ions have an effect similar to hard irradiation. Mustard oil, which is applied in the same manner as the mustard gas, has failed to show genuine mutagenic activity (VON ROSEN, 1954 b).

The investigations reported above on the mutagenic activity of the metal ions have proved that the radiomimetic reactivity, which is a typical reaction for complex-forming bivalent metal ions, is also associated with real mutagenic activity, as the author has already predicted in a previous paper (1954 b). The effect of the metals is comparable with the activity of ionizing radiation and known active chemical substances.

IV. DISCUSSION

As it is experimentally established that the metals in the cell are fixed in chelates or complexes similar to chelates, there may be more than one possible explanation for the pattern of their biochemical-physical reaction. The radiomimetic and mutagenic activity is characteristic of all those kinds of metals which have a complex-forming activity with nitrogenbases. Those complex-forming metals which at the same time have a high atomic number are, in extremely low concentrations, very poisonous in the cell metabolism. Among the active metals with a low or moderate atomic number members of the most essential »micro-elements» are represented. In a normal biochemical balance of the cell process the total content of some of these substances in the cell, as *e. g.*, Cu and Co, is extremely low. When the concentration in the cell increases over a fixed threshold value range, which seems to be allowed to vary ± 0.5 log mole/lit, then in the first stage weak poison symptoms as *e. g.*, arrested mitosis and other reversible narcotic cell effects, appear, caused by reversibly disturbed cell syntheses through breaking of valency bonds. By some higher dose concentrations irreversibly blocked or rearranged enzyme syntheses arise which sometimes lead to radiomimetic and mutagenic reactions. Still higher concentrations, that is a real overdosing, results in irreversibly broken down enzyme syntheses which bring about cell pycnosis and cell lethality. An increasing deficiency of a metal element may in principle cause the same stages of cell reactions.

After what has been stated above, therefore, the balance must be extremely delicate between the vitality of the cell system of the individual and an overdosage or insufficient dosage of a physical or chemical reactive phenomenon or agent. These hypothetical relations may be pictured (Fig. 6) in the following way: a narrow zone with both very weak overdosage and very weak insufficient content, where nevertheless the cell's vitality is optimal, is situated around an optimal concentration area in the cell's usual content of basic elements. The whole of this

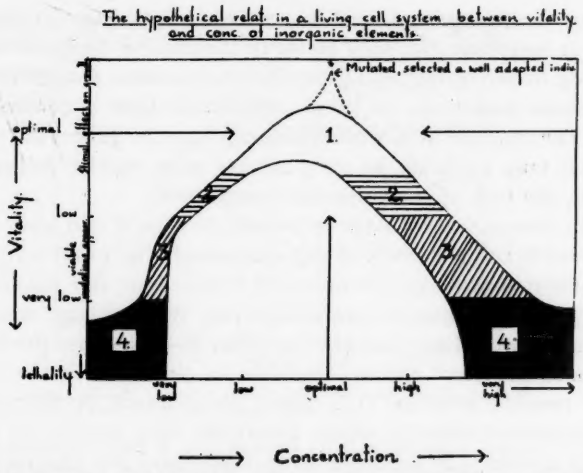

Fig. 6. The hypothetical relations in a living cell system between vitality and concentration of inorganic elements. For explanations see the text.

vitality area (zone 1) is characterized by a *balanced enzyme synthesis*. In the centre, however, a very narrow zone exists within which it ought to be possible for individuals with increased vitality reactions to be reproduced. Only this narrow zone, after mutation has occurred and the pressure of selection has acted on the altered genotype, is potentially capable of bringing forth individuals which are well adapted and therefore can react positively to the prevailing environment. In other words, in a universally applicable environment only extremely few individuals with a rearranged enzyme synthesis of the original type can exceed the optimal vitality.

Below the balanced enzyme region a somewhat unbalanced layer is to be found, which is caused partly by overdosage and partly by insufficient content. Here (zone 2) *reversibly disturbed syntheses* are obtained. These are caused by environmental conditions which have changed unfavourably to the organism, *e. g.*, altered reaction figure (P_H) in acid or too highly alkaline soils respectively, drought, changes in temperature, variations of the nutrient's content, etc. No actual radiomimetic effect or mutations result from such physiological disurbances. The lowering of vitality with insufficient dosage ought to be considerably more restricted than with overdosage.

After an indistinct transition the next zone is then reached, comprising

very high overdosage and very low content. Here (zone 3) *irreversibly blocked or rearranged valency bonds in the enzyme synthesis arise.* The result *may* under certain conditions be chromosome fragmentation and even genuine mutations. In these individuals there is generally a low vitality. The reaction area with overdosage may be quite wide while that for deficit may probably be very narrow with rapidly falling vitality caused by the lack of some essential compound.

Finally, extreme overdosage is present in zone 4 and also extremely low content to complete lack of any compound. The result is *irreversibly broken-down enzyme syntheses which bring about cell pycnosis and a lethal effect.* The region of overdosage may be very large while for insufficiency it would be somewhat smaller. In both cases the symptoms lead to a lethal effect for the organism in question. All the calculated areas of reaction overlap. This causes, for instance, further restriction of the important zone 3, where mutations may appear. It is indeed generally known, for example, how significant for the result of an ionizing radiation is the vitality of the material, the metabolic status (water content etc.), the power of resistance typical of its species, etc.

From the above arguments concerning the hypothetical relationships in the living cell between the vitality and the enzyme concentration on the one hand and the basic elements on the other, it will perhaps be obvious how important the cell vitality (=a balanced enzyme process) or a poison effect must be. Principally on the basis of the results gained from the experiments with metals the author calculated the hypothetical curves which are reproduced in Fig. 7. It records the curves of relationships between different concentrations of metals and the vitality of the cell. It will be seen that the complex formation phenomenon clearly has a certain relationship with that combination. The group in the metal system which embraces the basic elements on the cation side inevitably necessary to the plant (the so-called »nutrients», above all the alkali metals) has practically no chelate-forming disposition. The anion-forming elements, *e. g.* H, O, C, N, P, S, etc. must be judged by other backgrounds of comparison, because they react in the cell via other reaction mechanisms (*e. g.* the redoxsystem, the carbonium ion mechanism, the cysteine system etc.). The curve for the nutrient group is characterized by a broad maximum without a pronounced peak and while its legs fall shortly towards a lethal effect with total insufficiency or maximal overdosage.

The group of more or less weak complex-forming metals as Mg, Ca, Mn and others have a pronounced effect on the growth of the plant and

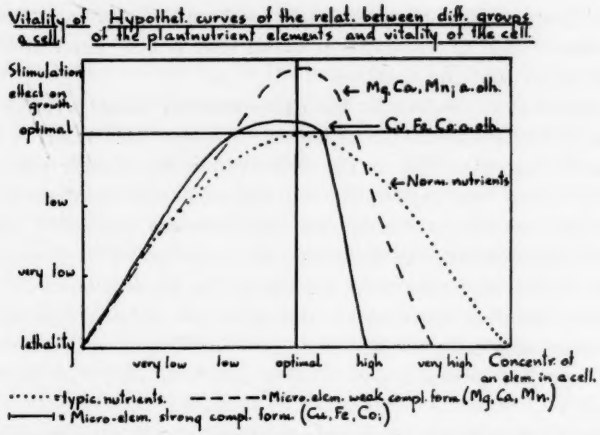
Vitality of a cell
Hypothet. curves of the relat. between diff. groups of the plantnutrient elements and vitality of the cell.
Stimulation effect on growth
optimal
low
very low
lethality
very low
low
optimal
high
very high
Concentr. of an elem. in a cell.
← Mg, Ca, Mn; a. oth.
← Cu, Fe, Co a. oth.
← Norm. nutrients
·····= typic. nutrients.
– – – – = Micro-elem. weak compl. form (Mg, Ca, Mn)
——= Micro-elem. strong compl. form. (Cu, Fe, Co;)

Fig. 7. Hypothetical curves of the relations between different groups of the plant nutrient elements and vitality of the cell. For explanations see the text.

specially Ca on the cell elongation. They would be characterized by a curve with a high peak at optimum concentration and more steeply falling legs down to lethality at extreme concentrations. The strong complex-forming metals which include the group of typical »micro-metal-elements» would form a curve with a low but clearly observable peak and then very steeply falling legs. Even very small variations of the concentration of such a metal ion in the cell will then be catastrophic for the living organism. In this case those metals which by a constant P^H always have the higher complex stability constant in the produced chelate may form a chelate and put out a metal with a lower constant. The observed results from the radiomimetic investigations do not favour this explanation, while the radiomimetic activity of the single elements is not additative, but nevertheless in some way specific.

A second theoretical suggestion would be that the complex-forming metal ions draw out chelate compounds from the formed nucleotides or nuclein acids forming stronger chelates in the plasma and thus inducing chromosome breaks. However, it has been observed that the optimal frequency of the chromosome breaks appears somewhat later after treatment with metal ions than after treatment with ionizing radiation and hard irradiation. Very few typical poisoning symptoms are noticed after treatment with ionizing agencies in contradiction to the observations after metal treatment. These differences indicate that the

activity of the metals must occur early in the nuclei acid synthesis. The chromosome breaking reactivity of metal ions is also much lower than the reactivity of ionizing radiation.

STEFFENSEN (1953 and 1955) has demonstrated induced radiomimetic effects in microspores of *Tradescantia* through artificially produced deficit in the plant of Mg or Ca ions. Above the author has already pointed out that treatment with Ca, Mg and also with some typical radiomimetic metals as Zn or Mn produces an increased luxuriant growth of young plants. All these metals are included in important cell processes. There were also weak effects on the growth observed (especially in the additative experiments) of Cu, Fe, Co and other metals which are known as necessary »micro-elements», while typical »macro-metal-elements» do not produce any vitality effect at all (VON ROSEN, unpublished).

The author therefore puts forward a third possible suggestion to explain the action of the metals in the cell. According to this they may react with the nucleosides or with the simplest nitrogen bases as *e. g.* aminoacids in the plasma. At overdosing they would form together abnormal chelates with high stability constants and thus bind necessary chelates which had to be used in the balanced cell synthesis later on. On deficit of specific metal ions free radical chelates may disturb the normal balance of the other and bound metals by substituting specific abnormal metal chelates. The same complications in principle may also occur, when the content of chelates or complexes similar to chelates, *e. g.*, EDTA, ribonuclease, ATP, hormones and others are subjected to variation in the cell. In all cases cell disturbances occur and radiomimetic and mutagenic effects may be induced. Let us consider the formulation of a general chelate principle, which is given *e. g.*, in BURSTRÖM and TULLIN, 1957, for the balance and state of bivalent complex-forming metals in the plant. According to this principle these metals in active states and during their migration occur in the form of chelates with various natural chelates as forming agents. There is in the cell a balance system between several metals (bound in chelates or free as ions) with different degrees of complex constants and also between several kinds of different attractive chelate compounds. Together these two kinds of biochemical systems form a reversible chemical balance; *e. g.* of the following equation:

$$Chel_1 + Me^{++}{}_2Chel_2 + Me^{++}{}_1 \rightleftarrows Me^{++}{}_1Chel_2 + Me^{++}{}_2Chel_1,$$

provided that the stability constants are:

$$Me^{++}{}_1Chel_2 > Me^{++}{}_2Chel_2 > Me^{++}{}_2Chel_1$$

Now it is also known that the complex constants change with the P_H-value and thus quite new balance conditions may occur from too great a variation of the P_H in the cell, above all by a decreased P_H. In some cells of *e. g.* fruits, a low P_H is observed, while it is assumed that the P_H in a generative cell or in another typical nucleus-dividing kind of cell normally lies close around P_H 6—7. If the P_H in a cell is shifted, changes of the »isoelectric point» (IEP) may occur in the protein gels and thus cell poisoning and further radiomimetic effects may be produced. This has already been demonstrated by the author (comp. VON ROSEN, 1954 b) by treatments with acids, acid salts, alkalis and halogens, which may be both active by themselves and through the induced acidity. By such physical treatments as temperature shocks, chromosome breaks are also induced. Another effect of the shifted P_H in a cell would be interruption of the system of balance between metal ions and chelates. As mentioned above, the result then may also be radiomimetic and mutagenic effects.

Of theoretical interest is the observation by the author (ibid. ref.) that even charged ions of non-complex-forming kinds in an electrolyte induce typical disturbances. This activity must be assumed to be a kind of ionizing treatment. The primary effect is poisoning of the cell metabolism which very often prevents the observation of the chromosomal or mutagenic activity. This complication by treatment with metal ions seems to prove the observed low optimum value to be about (c:a) 15 % frequency of cells with chromosome disturbances.

The last alternative may very well be extended to form a principle for the different abnormal reactions of metal ions in the cell processes. The author would prefer to suggest that in cells with normally balanced synthesis of the nucleotides and nucleic acids complex-forming metal ions keep together necessary simple amino-, amido and other compounds and thus substitute those chelates which together with ribos form the nucleosides. On their side these together with phosphoric acid substitute nucleotides, which later on polymerize to the end product DNA.

It is not yet known if complex-forming metals are able as a joint medium to form an integral part of the single nucleotides or also of the polymerized end product DNA. It has been demonstrated that they are included in several kinds of chemical, structurally known enzymatic compounds, and these seem to have very important functions. If a living plant were forced to absorb too great a content of a complex-forming »macro- or micro-metal-element», the result would be disarranged cell

syntheses by fixing necessary chelate parts (*e. g* nitrogen bases) in exceptional, substituted compounds or altering their normal frequency in the cell. The normal constituents of the DNA synthesis or enzyme synthesis are then not available for disposal in the cell and the normal synthesis is blocked. This would cause poison symptoms, chromosome disturbances and, as is demonstrated in these experiments, sometimes also real gene mutations. Even pure organic chelates are able to produce radiomimetic disturbances, as is demonstrated by McDONALD and KAUFMANN, 1957; HYDE, 1956; MAZIA, 1954 or WOLFF and LUIPPOLD, 1956 for EDTA, ribonuclease and other chelate compounds as for different kinds of hormones. The observations from the experiments by STEFFENSEN (1953 and 1955) may be explained as the reaction of free chelates, which have not been in balance with the cell metabolism.

TABLE 3. *Per cent disturbed cells from treatment with Me-nitrate or Me-EDTA. Root-tips of Pisum abyssinicum.*

Log. mole	9	8	7	6	5	4	3	2	1
$Fe^{III}\ NO_3$					0	0	2–6	2 (P)	P
EDTA		2	0	0	0	0	5	P	P
$Cu^{II}\ NO_3$		0	$\geqslant$1	5	2–9	$<$1–10	12	P	P
EDTA	0	0	1	0	0	0	3	P	
$Co^{II}\ NO_3$				$\geqslant$0	3	1–5	5–8	(P)	P
EDTA		0	0	0	1	2	1	14	P
$Zn^{II}\ NO_3$				$\geqslant$0	3	1–4	0–4	7	P
EDTA		0	0	0	1	1	6	P	
$Mn^{II}\ NO_3$						$\geqslant$0	5	4	(P)
EDTA		0	1	1	0	2	1	1	P
$Ca^{II}\ NO_3$						$\geqslant$0	2	4	3
EDTA		0	1	0	1	1	1	P	
$Mg^{II}\ NO_3$						0	0–1	2	2
EDTA			0	0	0	2	1	P	
$Na^{I}\ NO_3$						0	0	0	
EDTA				1	2	2	0	0	(P)
$H^{I}\ NO_3$				3	8	4	P	P	
EDTA					0	1	2	1	0

P=cell pycnosis

A recently performed cytological experiment by the author gives an illuminating view of the differences concerning the real concentration of the metal ions outside and inside the plant tissue. Root-tips of *Pisum abyssinicum* were treated with different concentrations of several more or less complex-forming metals, treated both as salts of nitrate and as EDTA-compound. The result of the cytological analysis is demonstrated in Table 3. It shows that in both kinds of metal compounds the optimum frequency of abnormal cells is produced by the same concentration of treatment solutions. The effect of EDTA itself is also to be shown in those metal chelates which contain a weak or non-complex-forming metal, *i. e.* in the lower part of the Table.

A $MeNO_3$ salt is fully dissociated in the P_H ranges about 7. The dissociation of the Me-EDTA is specific for each metal chelate and is far less for the strong complex-forming metals, as *e. g.* Fe, Cu, than for weak complex-forming ones, *e. g.*, Mn, Ca, Mg. The stability constant of the chelate is very sensitive for variations in the range of P_H. If, taking into consideration the position of the biochemical science to-day, one may be allowed to assume that the possible variation of the P_H in a living cell with a balanced mitotic activity would not be more than ± 2 P_H step, it would consequently follow that the principal content of dissociated free metal ions in such a kind of cell would be extremely low. Table 4 demonstrates the values of PM that is $-\log[Me^{++}]$ (=the same kind of information as for the H^+ ions in P_H), of the content of metal ions in a metal solution at the optimal radiomimetic concentration

TABLE 4. *Principle of optimum concentrations of* (Me^{++}) *at maximum chromosomal aberration effect.*

Signed as PM = $-\log(Me^{++})$

	Me-EDTA			$Me-NO_3$
	P_{H5}	P_{H7}	P_{H9}	P_{H7}
Fe^{+++}	19	22	24	3
Cu^{++}	12	15	17	5×4
Co^{++}	10	13	15	3
Zn^{++}	10	13	15	2
Fe^{++}	(8)	(11)	(13)	?
Mn^{++}	7	10	12	5×3
Ca^{++}	5	7	9	(5×3)
Mg^{++}	3	6	7	(2)
Na^+	$\geqq 1$	$\geqq 1$	$\geqq 1$	2

(Versene: 10 % excess).

for $MeNO_3$ and Me-EDTA. The Table indicates that the concentration of free strong reactive metal ions in the Versene solution is very much lower than that in the nitrate solution. If we thus assume a relative stable P_H value in the living generative cell, it may therefore be assumed that the real content of active metal ions in such a cell is generally of just this size. Thus we at first may suggest that of metal salts of nitrate only very few of the dissociated metal ions in the treatment solution have a chance to enter into the cell tissue. The Me-chelate on the contrary is lipophile and thus more easily enters through the cell walls. If opinion to-day is right that most of the compounds in the plasma are kinds of chelates or complexes similar to chelates, we surely may postulate that the available concentration in the cell of dissociated metal ions of a strong complex-forming metal in point of principle will be as low as is shown in the Table. Thus the concentrations of metal ions which in reality are needed for producing those poison effects, which may be able to induce radiomimetic and mutagenic reactivity, must be much lower than the results indicated by the experiments with dissociated inorganic salts. Parallel with the diminishing concentration of metal ions through the formation of new kinds of chelates the dissociation of the Me-chelate may proceed and thus more and more metal ions be available for the reaction. Simultaneously, however, the increased concentration of liberated chelates (*e. g.*, EDTA) may either force the chemical reaction in the reversible direction by the influence of its own stability constant or force the forming of other kinds of Me-chelate. This process would alter the total balance of different Me-chelates in the cell and so again drive the reactions back to a direction of balance. *If a gene mutation is induced, irreversible rearrangements of e. g. aminobases or aminoacids in the nucleic acid occur which may change the metabolic process into a new kind of balance.* If not, the cell would quickly be poisoned and die.

Thus the author believes it possible to postulate, as a principle, that the concentration of free complex-forming metal ions needed for radiomimetic and mutagenic reactivity is very low. Consequently the total content of free metal ions in a normal living cell must be still lower and thus very sensitive to even small changes. All these »micro-metal-elements» discussed here are widely spread over the earth and are present here and there in the earth crust in greater or smaller areas in higher concentrations than generally occurring. When plants are individually able or are forced to take up abnormal quantities of such an element, the result may be catastrophic for the plant population

migrating there and also for those animals which feed too long on such an extreme area.

Under certain conditions gene mutations too may be produced in a plant population. The principal part of these mutations ought to be lethal, but a few combinations may have a positive selective value and be able to take up the competition, within the population and also a positive selective value between populations when micro- or macro climates may happen to change. Opinion to-day is that either the cosmic radiation or the normal irradiation from isotopic elements in the earth crust are frequent enough to explain the production of spontaneous gene mutations. The author puts forward the hypothesis that *complex-forming metal elements may have played an important role in the evolution of plants an animals.* It would be of interest to chart the occurrence in Nature of these kinds of elements and their frequency relations to different typical spontaneous mutations. Recently WILKINS, 1957, has demonstrated interesting indications of genetically based tolerance to lead toxicity in *Festuca ovina* plants, which grew on the tip of an old Welsh lead mine. The author himself has also started experiments to establish the possible specific qualitative difference in mutation rate between some members among typical mutagenic metal groups.

A conclusion which may be drawn from the discussion above is *that the mutagenic activity of the metal ions may be of interest even for plant-breeding work.* It may be assumed that this mutagenic agency will give the breeders a new instrument when creating new artificially induced mutants, useful in plant-breeding work. This agency is very favourable too for combining with ionizing radiations and other types of treatments. The author has recently been able to demonstrate (unpubl. data) in a cytological investigation that the frequency of cell disturbances in *Pisum abyssinicum* by a combined treatment of complex-forming mutagenic metals and X-ray radiation is additive. The average discovered of 15 % disturbed cells after treatment with a 5.10^{-3} mole/lit. solution of metal nitrates or metal EDTA is *added* to the different frequencies of abnormalities produced from the different strengths of X-ray radiation. The X_2 of this series of experiments will be judged on gene mutation effects next year.

SUMMARY

Simple metal ions may induce both radiomimetic effects and genuine gene mutations of the same type which occurs from ionizing radiation

and from treatment with some chemical agencies as *e. g.*, mustard gas. The main material during the experiments has been species of *Pisum*. The biochemical principle which lies behind these reactions is the complex-forming ability among those reactive bivalent metal elements. The author assumes that interruptions of the chelate formation in the cell synthesis form the real background to the observed activity of the metal ions. The possible role in the evolution of the plant- and animal kingdom and the probable value for plant-breeding of the mutation activity observed are suggested. A new field for mutation experiments may here be opened and the results must hitherto be judged as interesting and promising.

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SELECTION WITH REGARD TO THE SEX DIFFERENCE OF BODY WEIGHT IN MICE

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THE sex difference of body weight varies not only between, but also within animal species. HOWARD (1941) *e. g.* found differences between inbred strains of mice. Such differences may in some cases be of importance to animal breeders. SHAKLEE, KNOX and MARSDEN (1951) found a marked variation between families in turkeys, and pointed out, that turkey breeders want equal size in both sexes. The difference of body size may also in other cases have an influence on the economical result, *e. g.* in fur bearing animals.

The object of the selection experiment, which will be described in this paper, was to study the possibilities to alter the sex difference of body weight in mice (*Mus musculus* L.)

The experiment has been carried out at the Institute of Animal Breeding of the Royal Agricultural College, and the investigation has been made possible by grants from the Royal Academy of Agriculture. Further, I am much obligated to Dr. D. S. FALCONER and Dr. J. W. KING from the Institute of Animal Genetics in Edinburgh, who have placed a part of their material at my disposal. I am also indepted to Professor IVAR JOHANSSON for his stimulating interest. I whish to convey my thanks to my co-workers and especially to Mr A. STRAUTMANIS, who has been assisting me in this work for several years, and to Mr A. UDRIS, who has prepared the diagrams, and also to Mr K. SKJÖLDEBRAND, who at the start collected mice.

MATERIAL AND METHOD

The animals in the ancestor generation from which both strains in the experiment descended, were caught at different localities in the town and in the surroundings of Uppsala. The majority of the animals were of

the wild agouti-colored type, but some were obviously crosses between wild mice and mice from laboratory strains. Some were brown and had not the light belly, which is typical of the wild mouse. Some of the last mentioned mice were carrying the recessive gene for albinism. Totally, 32 males and 33 females were caught. Obviously, 55 belonged to the wild type, and 10 were probably crosses. The object was to get a rather heterozygous ancestor generation, and the collected material obviously well satisfies this demand.

The caught animals were put together in pairs principally at random, although the time when the animals were caught, perhaps had some influence. The animals in the litters, which were born from these matings, formed the parental generation in the selection experiment. Only litters containing at least two males and two females were accepted, and from each of these litters the biggest male and the smallest female were chosen for one strain (Mf), and the smallest male and the biggest female for another strain (Fm).

The animals had a particular mice-bread a d l i b. with additional wheat germs. The young were taken from their mothers 21 days after birth, and at the same time the males were isolated from the females. The animals have been kept in glass cages, never more than six in each cage.

The experiment was carried out mainly in order to study the variance of body weight between mature males and females, and therefore, the selection was made with regard to the weight at 90 days. The animals were also mated at this age.

In the Mf-strain, the object of the selection was to increase the weight of the males and to decrease the weight of the females. In the Fm-strain, the selection was made in the opposite direction, the object being to decrease the weight of the males and to increase the weight of the females. In order to avoid a rapidly increasing inbreeding, the selection was in the first five generations made only within litters, and not between litters. In the Mf-strain, the heaviest male and the lightest female from every litter, which contained at least one male and one female, were chosen to be parents for the next generation. In the Fm-strain correspondingly the lightest males and the heaviest females were chosen.

Each of the generations 1—5 includes on an average 30 males and 30 females. In a few cases, when the mating has not led to pregnancy, the animals have been mated more than once. In the generations 6—10, three groups were formed in each generation of the strain, and in each group five successive litters were included. In the Mf-strain the three

litters in which the quotient of the mean weight of the males to the mean weight of the females was greatest were chosen, and from every one of these litters the two biggest males and two smallest females were selected to be parents of the next generation. In the groups of the Fm-strain, conversely, the three litters with the relatively lightest males and heaviest females were chosen, and from each of these litters the two smallest males and two biggest females were selected for breeding. If the litter size always had been sufficient for a complete selection of this kind, the number of selected animals would have been 18 males and 18 females in every generation of each strain. Actually, about 15—16 males and females respectively, remained. In each generation the 15 first born litters were brought into the experiment.

In addition, the investigation includes the earlier mentioned material from the Institute of Animal Genetics in Edinburgh. A detailed account of this part of the material will be given in the following section of this paper. The animals have been weighed at an age of six weeks.

THE INFLUENCE OF THE ENVIRONMENT ON THE SEX DIFFERENCE IN BODY WEIGHT

The material from Edinburgh derives from an investigation with mice on different planes of nutrition. The animals in one group (49 females) have had »full diet» and the animals in another group (26 females) »restricted diet». Each female was mated with the same male twice. The litters were standardized to a size of four males and four females, and the animals were weighed at an age of six weeks. In the group on full diet the males weighed on an average 26.68 g and the females 21.72 g. The corresponding weights in the group on restricted diet were 20.60 and 19.08. Hence, in the latter case the difference is much smaller between males and females. The ratio between the mean weight of the males and the mean weight of the females is in the group on full diet 1.23 and in the group on restricted diet only 1.08. An analysis of the co-variance between the mean weight of the males and the mean weight of the females in the litters has been made. Figure 1 shows the regression of the weight of the males on the weight of the females between and within the groups on full and restricted diet. Table 1 shows the results of the analysis.

The regression of the weight of the males on the weight of the females is in the group on full diet 0.973 and in the group on restricted diet 0.939. The difference is not significant. Hence, in Fig. 1 the lines A—B

and C—D do not differ significantly with regard to their slope. However, between the groups the coefficient of regression is 2.295, and the corresponding regression line (K—L) differs significantly from the regression lines within groups ($P < 0.001$). Accordingly, the different feed supply has had a greater influence on the body weight of the males than

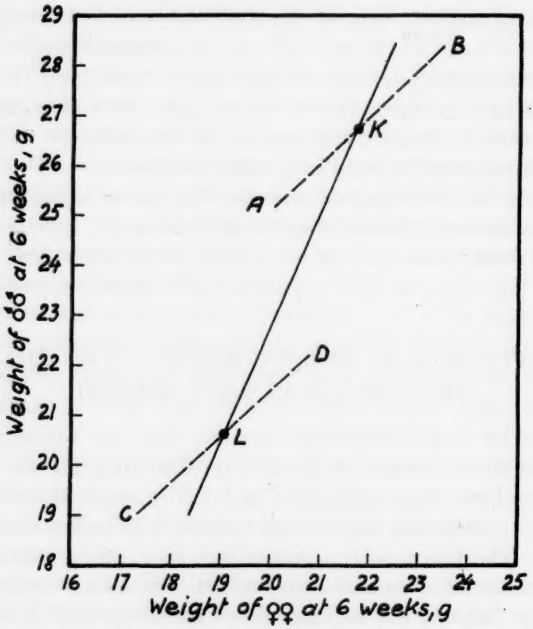

Fig. 1. Regression of the weight of the males on the weight of the females within groups on full (A—B) and on restricted (C—D) diet.

on that of the females. Probably, the greater growth capacity of the males could not be fully utilized in the group on restricted diet. BUTLER and METRAKOS (1950) have made the same observation, that environmental differences have a greater influence on the males than on the females. The authors found in their experiment with fostering mice of a female other than their mother that the males show the greatest decrease from fostering on an inferior milk supply. However, the significance of those observations has not been tested, and the material seems too small for safe conclusions.

The maternal influence on the difference in body weight has also been

TABLE 1. *Differences between a strain on full diet and a strain on restricted diet with regard to the lines of regression for the mean weight of the males on the mean weight of the females in the litter.*

Source of variance	D/f	Residual squares	Mean residual squares
Distribution of variates in relation to the total regression line (a)	148	535.14	
Distribution of variates in relation to the parallel regression lines within strains (b)	147	292.05	1.99
Error a—b = difference between the two strains in regard to their lines of regession	1	243.09	243.09
Distribution of variates in relation to the regression lines of each strain (c)	146	291.99	2.00
Error b—c = differences between the two strains in regard to the regression coefficients	1	0.06	0.06

Quotient: $\frac{243.09}{1.99} = 122.16$ ($P < 0.001$).

studied on the author's material from Uppsala. The correlations between parents and offsprings have been recorded in Table 2. The coefficients of correlation and regression have been calculated separately for the Mf-strain and the Fm-strain, and in both cases the coefficients are estimated within generations.

The correlation between parents and offsprings is in all cases stronger in the Fm-strain with the bigger dams and hence probably with the

TABLE 2. *Co-variance between parents (P) and offspring (O) within generations 1—10.*

	Mf-strain				Fm-strain			
	number of		r	$b_{O/P}$	number of		r	$b_{O/P}$
	litters	offspring			litters	offspring		
Dam-daughter	230	668	0.245	0.321	225	677	0.402	0.380
Dam-son	230	652	0.304	0.486	225	657	0.397	0.502
Sire-daughter	230	668	0.190	0.165	225	677	0.372	0.363
Sire-son	230	652	0.139	0.153	225	657	0.301	0.378

more sufficient pre- and postnatal environment, which favors the growth. The regression of the body weight of the offspring on the body weight of the sires will in fact be of entirely genetic origin. The heritability estimated according to the regression of the son on the sire ($h^2=2xb_{O/P}$) is in the Mf-strain 0.31 and in the Fm-strain 0.76. Because the difference between the strains with regard to the degree of inbreeding is small, the inbreeding coefficients have not been taken into consideration. However, the marked difference between males and females with regard to their body size and the fact that different selection has been practised in the strains, entail that coefficients of regression can not be very useful for estimating the heritability of the body weight. The coefficients of correlation are not influenced as much by those factors. The correlation between parents and offspring is in the Mf-strain 0.203 ± 0.047 and in the Fm-strain 0.363 ± 0.042. The heritability estimated according to the correlation between sire and son is in the Mf-strain 0.28, and in the Fm-strain 0.60. The corresponding heritabilities, estimated according to the correlation between sire and daughter are 0.38 and 0.74, respectively. The values are in both cases about twice as great in the Fm-strain as in the Mf-strain. The correlations between dam and offspring are in all cases greater than the corresponding correlations between sire and offspring. The correlation between dam and offspring, within strains and generations, is 0.348 ± 0.043, and the corresponding correlation between sire and offspring is only 0.251 ± 0.046. The correlation between dam and offspring is obviously not caused only by heredity. Environmental factors will be of importance too. The superior prenatal and postnatal environment, which the bigger mothers offer their offspring, seems to increase the correlation between the body size of the dam and the body size of the offspring. It will also be noticed, that the maternal influence seems to be greater on the sons than on the daughter. The differences between the coefficients of correlation between dam and son and that between sire and son are in both strains greater than the difference between the coefficient of correlation between dam and daughter and that between sire and daughter. Although the difference in this case is not significant, the result suports the earlier mentioned observation on the greater susceptibility of the males to inferior environment.

It may also be mentioned, that in both strains the correlation between sire and daughter is greater than that between sire and son. Further, in the Mf-strain the correlation between mother and son is greater than that between mother and daughter, and the conclusion may perhaps be

drawn, that there should be in the X-chromosomes genes which had influence on the body size. However, the phenomenon can be explained as a result of the greater environmental variance of the size of the males. This will decrease the correlation between sire and son in comparison with the correlation between sire and daughter. And the greater susceptibility of the males will also increase the correlation of maternal origin between dam and son in comparison with that between dam and daughter. In the Fm-strain the maternal environment is perhaps in all cases nearly satisfactory, and so the observed special influence of differences in the maternal environment does not work to any extent in this case. The correlation between dam and daughter in this strain is practically as strong as the correlation between dam and son. Even if there would be a small amount of special maternal influence on the sons, the greater susceptibility of the males would decrease the last mentioned correlation.

SELECTION FOR SMALLER AND GREATER SEX DIFFERENCE OF BODY WEIGHT

In order to avoid a rapidly increasing inbreeding the selection was in the first five generations made only within litters and not between litters. However, in the generations 6—10 selection has also been made with regard to the difference between litters. The coefficients of inbreeding were estimated for the individuals in the 5th and in the 10th generation. The averages in the two strains were:

5th	generation,	Mf-strain	0.11
»	»	, Fm-strain	0.03
10th	»	, Mf-strain	0.15
»	»	, Fm-strain	0.10

Tables 3 and 4 and Fig. 2 shows the selection intensity and the results.

Table 3 shows the ratios between the mean weight of the males and the mean weight of the females in the litters. The table shows also the ratios between the weight of the sires and the mean weight of their female litter mates, and between the mean weight of the male litter mates of the dams and the weights of the dams. The last mentioned ratios in the parental generations have also been used in Fig. 2 as a measure of the selection intensity. The results of the selection have been measured by the ratio between the mean weight of the males and that of the females within litters.

Table 4 gives some data on the litters in both strains in ten gener-

TABLE 3. *Ratios betweeen the weights of males and females.*

Strain and generation	Number of litters	Ratio: weight of the sire to av. weight of the sisters of the sire (a)	Ratio: av. weight of the brothers of the dam to the weight of the dam (b)	$\frac{a+b}{2}$	Litters in which the sires are born: Ratio: av. weight of males to av. weight of females (c)	Litters in which the dames are born: Ratio: av. weight of males to av. weight of females (d)	$\frac{c+d}{2}$	Ratio: av. weight of males to av. weight of. females in the litter
Mf:								
1	27	1.22	1.18	1.20	1.16	1.11	1.14	1.18
2	28	1.25	1.27	1.26	1.18	1.20	1.18	1.22
3	32	1.34	1.27	1.31	1.24	1.20	1.22	1.20
4	33	1.27	1.33	1.30	1.23	1.20	1.22	1.22
5	30	1.43	1.37	1.40	1.32	1.24	1.28	1.20
6	20	1.31	1.34	1.33	1.31	1.34	1.33	1.19
7	15	1.38	1.33	1.36	1.32	1.34	1.33	1.22
8	15	1.31	1.35	1.33	1.33	1.35	1.34	1.28
9	15	1.47	1.31	1.39	1.41	1.31	1.38	1.26
10	15	1.41	1.39	1.40	1.42	1.36	1.39	1.23
Fm:								
1	30	1.06	1.05	1.06	1.14	1.12	1.13	1.22
2	30	1.09	1.06	1.08	1.20	1.18	1.19	1.17
3	30	1.06	1.05	1.06	1.16	1.16	1.16	1.21
4	30	1.09	1.07	1.08	1.18	1.18	1.18	1.19
5	30	1.07	1.11	1.09	1.18	1.18	1.18	1.12
6	15	0.97	0.91	0.94	0.98	0.96	0.97	1.18
7	15	1.12	1.07	1.10	1.09	1.07	1.08	1.10
8	15	0.98	1.02	1.00	1.00	1.02	1.01	1.15
9	15	1.06	1.08	1.05	1.05	1.06	1.06	1.15
10	15	1.00	1.13	1.07	1.05	1.05	1.05	1.17

ations. This table shows *e. g.*, that the average weight at 90 days has been almost unchanged in both strains in all generations. However, in the later generations of the Mf-strain, there is a small tendency for decreasing body weights, which is probably due to the increasing inbreeding. The tendency mentioned, is smaller in the Fm-strain.

Table 3 and Fig. 2 show, that the result of the selection is two populations, which differ with regard to the sex difference of body weight. An analysis (Table 5) shows, that this difference between the strains is clearly significant ($P < 0.01$). The figure also shows that the difference between the strains has increased during the selection experiment. The

TABLE 4. *Litter size and average weights of males and females in the different generations of the*

TABLE 4. *Litter size and average weights of males and females in the different generations of the Mf- and Fm-strains.*

Strain and generation	Number of litters	Females					Males				
		Number per litter	Mean weight g	Between litters σ	Within litters σ	Within litters var. coeff.	Number per litter	Mean weigt g	Between litters σ	Within litters σ	Within litters var. coeff.
Foundation generation	30	2.97	16.2	2.36	1.26	7.8	2.77	18.4	2.03	1.42	7.7
Mf-strain											
1:st generation	27	3.19	16.8	2.23	1.26	7.5	2.78	19.9	2.36	1.83	9.2
2:nd »	28	2.32	17.9	1.86	1.73	9.7	3.04	21.8	2.54	2.23	10.2
3:rd »	32	3.22	18.5	1.51	2.37	12.8	3.03	22.2	1.94	2.47	11.1
4:th »	33	3.12	18.1	1.16	2.09	11.5	2.97	22.1	2.55	2.62	11.9
5:th »	30	2.97	18.0	2.50	1.58	8.8	2.53	21.6	2.36	2.09	9.7
6:th »	20	2.70	18.9	1.20	1.90	10.1	2.80	22.5	1.07	2.53	11.2
7:th »	15	2.73	18.2	1.88	1.91	10.5	2.33	22.2	1.74	2.15	9.7
8:th »	15	2.73	17.4	1.72	1.92	11.0	3.07	22.2	1.53	2.68	12.1
9:th »	15	2.67	17.1	1.56	1.31	7.5	2.73	21.5	1.57	2.28	10.6
10:th »	15	3.07	16.6	1.30	1.65	9.9	2.87	20.4	1.74	2.47	12.1
Fm-strain											
1:st generation	30	3.10	17.0	1.90	1.59	9.4	3.40	20.7	2.01	2.63	12.7
2:nd »	30	3.57	18.9	2.17	1.99	10.5	3.20	22.1	2.86	2.73	12.4
3:rd »	30	2.83	18.2	1.82	2.01	11.0	3.53	22.0	3.30	2.18	9.9
4:th »	30	3.23	18.4	2.34	2.51	13.6	3.13	21.9	2.43	1.85	8.4
5:th »	30	3.27	19.4	2.53	2.37	12.2	2.70	21.7	2.50	2.41	11.1
6:th »	15	2.60	18.5	2.06	1.55	8.4	2.60	21.8	2.48	3.08	14.1
7:th »	15	2.87	18.9	1.88	2.01	10.6	2.47	20.7	2.36	2.53	12.2
8:th »	15	2.87	17.9	1.52	1.98	11.1	2.07	20.6	2.07	2.54	12.3
9:th »	15	2.80	18.2	1.14	2.35	12.9	2.13	20.9	1.59	2.33	11.1
10:th »	15	2.00	17.8	2.32	1.71	9.6	2.60	20.9	1.32	3.70	17.7

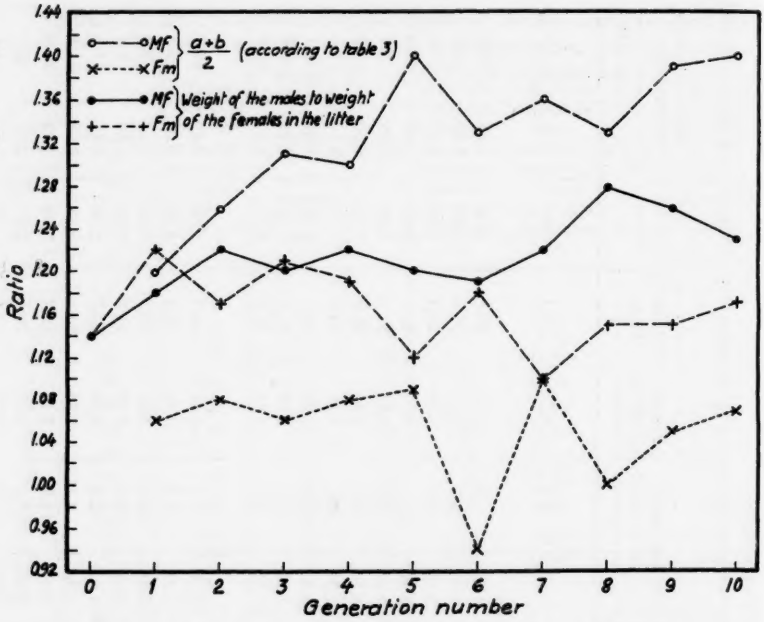

Fig. 2. Selection for large and for small sex difference of body weight.

coefficient of correlation (if the correlation is estimated as linear) between the order number of the generation and the differences between the two strains shown in Fig. 2, is 0.712 ($P < 0.03$).

It may also be noticed, that the sex difference of body weight, estimated as the average of both strains is the same in the five first generations and in the five latter ones. The average ratio between the weight of the males and the weight of the females is in both cases 1.193. But, as previously mentioned, the difference between the strains has increased.

The environmental influences, which have been discussed in this paper, have also to be taken into account by studying the results of the selection. The aim of the selection is in the Mf-strain to get bigger difference between the body weight of the males and females. Therefore, in this strain small females have been chosen for breeding. The pre- and postnatal environment of the offspring will be inferior, and the growth rate of the male offspring will be more influenced and retarded than

TABLE 5. *Analysis of the variance of the ratio between the weight of males and the weight of the females.*

Source of variation	D/f	Sum of squares	Mean square
Total	19	0.03582	
Between the strains Mf and Fm	1	0.01458	0.01458
Within the strains Mf and Fm	18	0.02124	0.00118

Quotient: $\frac{0.01458}{0.00118} = 12.36$ ($P < 0.01$).

the growth rate of the female offspring. Thus, this environmental effect will counteract the effect of the selection.

THE GROWTH OF MALES AND FEMALES IN THE STRAINS

In the generations 7—10 all animals have been weighed every 10th day. The first weighing was made at an age of 10 days and the last at an age of 90 days. Table 6 and Fig. 3 show the results.

The discrepancy between the weights noticed in Table 4 and the corresponding averages in Table 6 is caused by differences in the methods of calculating. In Table 4 the averages are computed from the mean weights in the litters. Every litter counts equally, independent of the number of offspring in the litter. The weights in Table 6 are averages of the weights of the single individuals. There is, as *e. g.* MACARTHUR (1949) has pointed out, a positive correlation between the litter size and the weight of the adult animals. In the case of this investigation the correlation between the litter size and the average of the mean weight of the males and the mean weight of the females is 0.13 ($P < 0.01$).

Table 6 and Fig. 3 show, that at an age of 10 days the males as well as the females are heavier in the litters in the Fm-strain. The dams are bigger and the environment probably more sufficient for the offspring. At an age of 50 days this difference still remains. Already, however, at an age of 40 days the males are growing faster in the Mf-strain than in the Fm-strain. Hence, the difference between the strains with regard to the difference between the weight of the males and the weight of the females appears about at the time of sexual maturity. Therefore, the growth during this period has been studied separately.

TABLE 6. *The weights in grams of the males and females during the growth period in the strain Mf and Fm.*

Age, days	Mf strain								Fm strain							
	Generation number								Generation number							
	7		8		9		10		7		8		9		10	
	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀
10	4.9	4.9	4.6	4.6	5.0	4.9	4.2	4.3	4.8	4.9	5.2	5.3	4.7	4.7	4.6	4.8
20	8.5	8.1	8.4	8.0	8.1	8.4	6.7	7.1	8.3	8.5	8.6	9.0	8.7	8.7	8.1	8.5
30	14.0	12.1	13.5	11.6	13.6	12.2	12.1	11.6	13.9	12.9	13.5	12.7	15.0	13.3	13.5	12.8
40	17.4	14.8	17.4	14.0	15.7	14.7	15.6	14.1	17.4	15.7	16.9	15.2	18.6	15.8	17.0	14.7
50	18.7	15.7	18.8	14.6	18.6	15.2	17.8	15.0	18.4	16.5	18.4	15.9	19.8	16.5	18.2	15.6
60	19.8	16.3	19.9	15.2	19.7	15.8	18.9	15.4	19.2	17.1	19.2	16.8	20.4	16.9	19.1	16.4
70	20.8	17.2	20.9	16.0	20.5	16.7	19.6	16.0	19.8	18.0	20.0	17.3	20.7	17.6	20.0	16.9
80	21.9	17.8	21.8	16.6	21.2	17.0	20.2	16.4	20.3	18.6	20.7	17.9	21.2	18.1	20.6	17.3
90	22.6	18.4	22.7	17.2	21.9	17.6	20.9	17.0	21.0	19.2	21.5	18.5	21.5	18.3	21.2	17.7

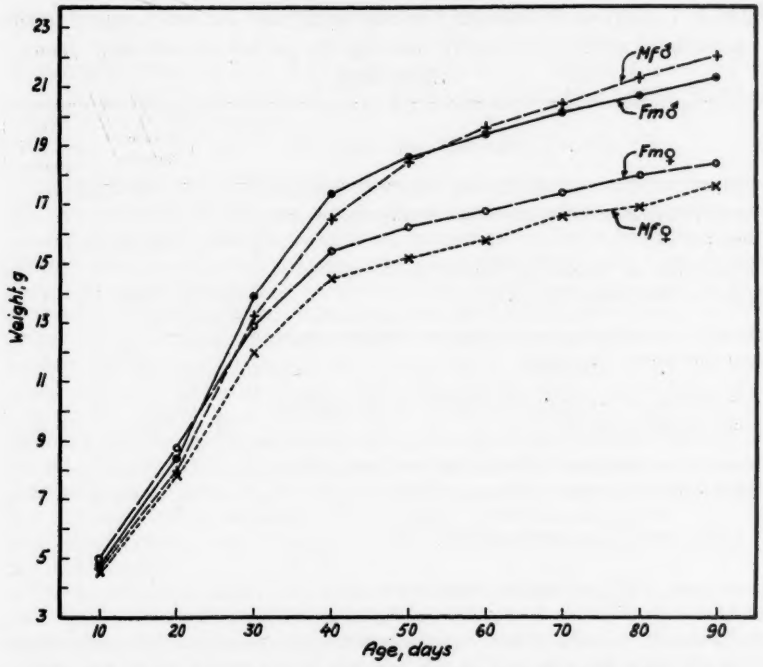

Fig. 3. Growth of the males and the females in the strains Mf and Fm in the generations 7—10.

The difference between the growth of the males and the growth of the females in the Mf-strain and the Fm-strain during the period from 30 days until 60 days has been studied in an analysis of co-variance (Table 7). The age (x) has been transformed into a logarithmic scale because the correlation between the weight and the logarithm of the age in this case seems to be nearly linear. The other variable (y) is the difference between the mean weight of the males and the mean weight of the females in the strain, generation and age group. It is evident, that not only the difference between the regression lines but also the difference between the coefficients of regression is significant ($P < 0.05$).

CONCLUSIONS

A study of the environmental influence on the growth rate of males and females has shown facts, which indicate a greater susceptibility of

TABLE 7. *Difference between the strains Mf and Fm with regard to the growth of males and females during the period 30—60 days from their birth.*

Source of variance	D/f	Residual squares	Mean residual squares
Distribution of variates in relation to the total regression line (a)	30	20.50	
Distribution of variates in relation to the parallel regression lines within strains (b)	29	17.56	0.61
Error a—b=difference between the two strains in regard to their lines of regression	1	2.94	2.94
Distribution of variates in relation to the regression lines of each strain (c)	28	15.07	0.54
Error b—c=difference between the two strains in regard to the regression coefficients	1	2.49	2.49

Quotients: $\frac{2.94}{0.61} = 4.82$ ($P < 0.05$).

$\frac{2.49}{0.54} = 4.61$ ($P < 0.05$).

the males. A restricted feed supply retards the growth of the males more than the growth of the females.

A part of the variation of the sex difference of body weight is of hereditary origin, and selection for smaller or bigger difference between the sexes alters the population with regard to this difference.

The difference between the growth rate of males and females in strains, which have been reversely selected for the sex difference of body weight, seems to be manifested mainly at the time when the animals reach maturity, *i. e.* at an age of 30—60 days.

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BRIEF REPORTS

H. SJØSETH: Studies on frost hardiness in diploid and autotetraploid red clover (*Trifolium pratense*) and winter rye (*Secale cereale*).

(Received May 10th, 1957)

The hardiness of experimentally produced polyploids has hitherto been but little investigated. Studies on the geographical distribution of naturally occurring polyploids, have shown that the polyploids are more frequent in arctic climates than their diploid relatives. (HAGERUP, 1932, MÜNTZING, 1936. FLOVIK, 1940, LØVE and LØVE, 1943). According to the conclusions drawn on the basis of these investigations, it has been suggested by several workers that the polyploids are more hardy than the related diploids. The investigations hitherto carried out on frost resistance in experimentally produced polyploids have given somewhat different results. Thus, SCHLÖSSER (1936), PIRSCHLE (1941) and MYERS (1947) have reported that artificially produced polyploids exhibited a greater sensitivity to cold injury than the corresponding diploids. On the other hand, NISHIYAMA (1934) and KOSTOFF (1938) have observed that the polyploids are superior to their diploid relatives in this respect. BOWDEN (1939) and NIELSEN (1947) working with naturally occurring polyploids, have not been able to observe any clear correlation between winterhardiness and chromosome number.

The present paper deals with some preliminary results regarding frost resistance in diploid and colchicine-induced autotetraploid material of red clover (*Trifolium pratense*) and winter rye (*Secale cereale*). The investigations are carried out in the laboratory at the Institute of Genetics and Plant Breeding, Agricultural College of Norway. A more detailed report on the frost resistance experiments particularly in diploid red clover is given by the author (SJØSETH, 1957).

Red clover

The red clover material for the present study comprises 4 diploid strains or selected families of Norwegian late red clover, and 7 autotetraploid families which originate from the same or nearly related material. Furthermore, 6 diploid and autotetraploid F_1 families, arisen from chimera plants, have been tested in freezing experiments. The material of winter rye consists of diploids and autotetraploids of the three varieties Grårug, Kungsråg II and Bjørnråg.

The artificial freezing tests were carried out in this way: The plant material was grown in wooden boxes with a definite number of plants per box. In each box all the varieties were distributed at random. About three months after seeding the plants were stored in a hardening room at a temperature of $+1.5^\circ$ C for two weeks before applying freezing treatments. The material was frozen partly in a refrigerating chamber and partly in large test tubes in a refrigerating cabinet. In the refrigerating chamber the red clover plants were exposed to a temperature of about -9° C for 48 hours, while those treated in test tubes, at -8° C for three hours. The rye material was treated in test tubes at -12 to -16° C for five hours. After the freezing tests the plants

were thawed slowly and stored at greenhouse temperature for three weeks, whereupon the number of surviving plants was estimated.

A review of the results of freezing tests in red clover is given in Table 1.

TABLE 1 a. *Freezing experiments on diploid and autotetraploid red clover (Trifolium pratense) 1954 to 1957.*

Series	No. of experiments	No. of strains or families		No. of plants per strain frozen	Average percentages of surviving plants		F-values		
		2x	4x		2x	4x	Within 2x	Within 4x	Between 2x and 4x
I	6	3	2	1100	36.1	22.4	15.87**	—	21.75**
II	5	3	3	1100	51.1	30.0	109.24***	13.38**	88.27***
III	6	2	3	1500	40.4	25.4	77.35***	95.72***	33.23**

TABLE 1 b. *F_1 families arisen from chimera plants.*

I	3	3	3	360	58.9	32.6	—	4.5*	33.18**
II	1	3	3	120	42.9	20.5	33.32**	16.13**	110.56***

The comparison of the frost resistance in diploids and tetraploids is made on the average percentage of surviving plants within each group. A fact of essential interest in Table 1 is the highly significant difference between diploids and tetraploids in this respect. In all the series of experiments the tetraploids prove to be considerably more sensitive to frost injuries than the diploids. In most cases there are significant differences within diploid and tetraploid groups.

The 2x and 4x groups in Table 1 a are not strictly comparable as to their genetic origin, but comprehensive field tests of a large number of strains of Norwegian late red clover have shown little, if any, differences in winter killing. It seems therefore safe to conclude that the large differences found between the chromosome groups are due to the difference in chromosome number. The results obtained with F_1 families (Table 1 b) give a further proof of this conclusion. In this material the 4x group represents a doubling of the gene mass of the diploid and, allowing for segregations from crosses of F_1 plants and families, the pairs of 2x and 4x families should, on the average, contain the same gene complements.

A significant interaction polyploidy × variety was not established in this material. However, one of the tetraploid families in series III, Table 1 a, descending from a very hardy diploid included in this test, was found to be much more frost resistant than the other two tetraploid families, but less frost resistant than the corresponding diploid. After 6 freezing experiments the average percentage of surviving plants were 48.5 for the diploid family, while 41.1 for the corresponding tetraploid, the difference being significant at the 5 percent level. This emphasizes the importance of the genotype of the diploid for the results obtained by chromosome doubling, and

indicates that sufficiently hardy tetraploids may be obtained on the basis of hardy diploids.

Winter rye

The results obtained in freezing tests in winter rye (Table 2) prove to be closely similar to those obtained in red clover.

TABLE 2. *Freezing experiments on diploid and autotetraploid winter rye (Secale cereale) 1956.*

Varieties	No. of experiments	No. of plants per variety frozen	Average percentage of surviving plants		F-values			
			2x	4x	Within 2x	Within 4x	Between 2x and 4x	Interaction polyploidy × variety
Grårug	4	1000	47.8	34.8	5.70*	11.67**	78.86**	7.83**
Kungsråg II	4	1000	37.6	26.9				
Bjørnråg	4	1000	49.3	21.8				

The differences in frost resistance between diploids and tetraploids is found to be highly significant in favour of the diploids. There are also significant differences between varieties within the diploid and the tetraploid groups. Furthermore, there is a significant interaction polyploidy × variety, the varieties have reacted differently on chromosome doubling.

The rye material was frozen when the plants had obtained two to three leaves. Plants treated at a later stage of development, not being included here, showed but little cold injury even at a freezing temperature of —15 to —16° C. Under these conditions the difference between diploids and tetraploids was less reliable.

The results referred to in this study indicate that the frost resistance, to a certain extent, is reduced by chromosome doubling. The tetraploid strains showed, however, differences in this respect, and the results indicate that relatively frostresistant tetraploids may be obtained by the doubling of highly frost resistant diploids.

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ARNE MÜNTZING: Frequency of accessory chromosomes in rye strains from Iran and Korea.

Though the typical chromosome number of rye, *Secale cereale*, is $2n=14$, individuals with additional accessory chromosomes of a specific type are known to occur in rye populations from various parts of the world. When such chromosomes are present, their number is generally two or more rarely four, plants with uneven numbers (one or three) being quite rare. This preponderance of even numbers of accessory chromosomes is caused by the occurrence of a process of directed non-disjunction, occurring at the first pollen mitosis and at the corresponding stage on the female side (*cf.* MÜNTZING, 1954).

Some commercial varieties of rye seem to be quite devoid of plants with accessory chromosomes; in other varieties they may be present in low frequencies. In more primitive strains of rye from Turkey, Afghanistan and Transbaikal accessory chromosomes were, on an average, found to be more frequent, though also in these regions populations may occur which entirely lack such extra chromosomes (*cf.* MÜNTZING, 1954, Tab. 1).

Thanks to the courtesy of Professor H. KUCKUCK, who spent several years in Iran, a comprehensive material of rye from this country could be examined with regard to the presence or absence of accessory chromosomes. The seed material kindly put at my disposal was from ears collected in Iran at 88 different localities, distributed over the country. Each locality was represented by several spikes (generally 5 to 10), taken from different individuals. The chromosome numbers were determined for each open pollinated spike progeny separately and then added to a total series, representing the population in question. As a rule the separate plant progenies were small and average differences in chromosome numbers between them were therefore difficult to establish. However, by adding the values of plants representing the same population more reliable figures could be obtained.

Chromosome counts were undertaken in a total of 3111 individuals, the

average number of plants examined per population being 35.4. For all these chromosome counts I am much indebted to my technical assistant Miss M. PALM. In 65 populations at least 20 individuals were studied, in the remaining 23 cases less than 20 individuals per population were available for cytological examination.

TABLE 1. *Occurrence of accessory chromosomes in rye populations in Iran.*

	Average percentages of plants with accessories in 88 populations											Number of populations
	0	1 — 5	— 10	— 15	— 20	— 25	— 30	— 35	— 40	— 45	— 50 — 55	
Values accurate	24	21	14	2	2	1	1					65
» approximate	15	—	2	1	—	—	1	1	1	1	1	23
Total	39	21	16	3	2	1	2	1	1	1	1	88

As may be seen from Table 1, 24 of the 65 populations accurately examined contained no accessory chromosomes at all, whereas in 41 populations plants with such chromosomes were present in frequencies ranging from 1 to 30 per cent. In 15 of the 23 less accurately examined populations no plants with accessories were found, whereas in the remaining 8 cases they were present in frequencies ranging from 1 to 55 per cent.

In some of the 15 populations the lack of accessories is probably only apparent. If a larger material had been available some of them would probably have been found to contain a low proportion of plants with such chromosomes. In the total series of 88 populations (Table 1) 49 were found to contain accessory chromosomes, which corresponds to 56 per cent of the populations. This is a minimum value, the real percentage being somewhat higher. Roughly, it may be estimated that *in Iran about two thirds of the rye populations contain accessory chromosomes.* However, in such populations the frequency of plants with accessories is in most cases fairly low (less than 10 per cent).

The Iran populations studied were very scattered and only represented test samples from a much larger material studied morphologically and genetically by Professor KUCKUCK. No correlation between frequency of accessories and geographical distribution could be traced, populations with and without accessories apparently being distributed at random and sometimes occurring side by side. In this connection it should be mentioned that Prof. KUCKUCK informed me that adjoining localities may often be effectively isolated from each other by mountain ridges.

In the populations containing plants with accessory chromosomes the number of accessories per plant was generally 2, plants with 1, 3 or 4 accessories being exceptional. This is evident from the following series, representing the sum of the chromosome counts in the 49 populations containing plants with accessories.

	Number of accessory chromosomes				
	0	1	2	3	4
Number of plants:	1812	14	137	1	4

Plants with two accessories were about ten times as frequent as plants with only one such chromosome. The preponderance of an even number of accessories is certainly caused by the occurrence of the usual non-disjunction mechanism. Such a mechanism was demonstrated to occur in several other rye strains, showing a similar distribution of accessories in natural populations (*cf.* MÜNTZING, 1954, Tables 1—2). The accessory chromosomes in the Iran populations were all of the usual standard type, characterized by a smaller size than the ordinary rye chromosomes and a subterminal position of the centromere.

Although accessory chromosomes are often found in the rye populations of Iran, there is another region in the Far East, in which such chromosomes are much more frequent. The work of OINUMA (1952) indicated a high frequency of accessories in rye from Korea, and this has now been verified, as may be seen from Table 2. The seven strains examined were raised from seed material most kindly put at my disposal by Dr. CHANG CHOON WOO of the National Institute of Horticulture, Pusan.

TABLE 2. *Number of accessory chromosomes in seven strains of rye from Korea.*

Field number	Place of origin	Number of acc. chr. 0	1	2	3	4	n	Percentage of plants with acc. chromosomes	Average number of acc. chr. per plant
030	Seoul No. 1	17	—	9			26	34.6	0.69
031	» No. 2	29	1	6			36	19.4	0.36
032	Suwon	7	3	16	1	6	33	78.8	1.88
033	Yonkii	2	2	9	—	6	19	89.5	2.32
034	Booyou	4	—	34	2	7	47	91.5	2.17
035	Kongjoo	25	1	14	1	3	44	43.2	1.00
036	Kyongsong-namdo	19	1	4			24	20.8	0.38

The results of the chromosome counts are, indeed, interesting, the average frequency of accessory chromosomes being much higher than in any other geographical region so far studied. This is especially true of the populations from Yonkii and Booyou, in which the average percentages of plants with accessory chromosomes were as high as 89.5 and 91.5 per cent respectively. The frequencies in question are significantly different in different populations, the lowest value, 19.4 per cent, being found in No. 031 (Seoul No. 2).

Also in these population the accessory chromosomes are of the standard type, but in a few plants some structurally deviating types were also observed. As usual, even numbers of accessories, 2 and 4, are predominant.

So far the surprisingly high concentration of accessory rye chromosomes in Korea is just a puzzling fact, and we do not know whether this concentration is correlated with special climatic and edaphic conditions in this part of the world, or whether it is only a consequence of historic factors during the distribution of the species *Secale cereale*. Under such circumstances a thorough

study of the rye populations in Korea and adjoining countries is, indeed, urgent. In the meantime investigations of the genetical and cytological effects of the accessory chromosomes in the populations already available may be undertaken. As the presence of accessory chromosomes seems to be a primitive trait studies of these chromosomes may be of importance for the problem of the centre or centres of origin of cultivated rye.

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